NATURAL SELECTION OF LIFE HISTORY TRAITS IN AN ESTUARINE AMPHIPOD

by

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Natural Selection of Life History Traits in an Estuarine Amphipod

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ABSTRACT

This thesis documents microgeographic variation in life history traits of an estuarine amphipod (*Eogammarus confervicolus)* in three habitat types (termed bank, wood debris and *Fucus*) within the same estuary. I show through reciprocal transplant experiments performed in laboratory simulated habitats and interpopulation crosses that the observed variation has a genetic basis. The crosses indicated dominance of many of the life history traits (including life span) in amphipods from wood debris and *Fucus* over those from bank. The absolute fitness of amphipods was greatest in their native substrate, as was their fitness relative to members of other habitat types, when raised in their substrate. Recombinant DNA techniques indicated the genotypes of each of these estuarine populations were distinct and provided markers for use in competitive ability experiments between life history types.

Further evidence of selection came from sampling additional populations from the same three habitat types and demonstrating a close correlation of life history traits with environment type. A laboratory selection experiment involving members of one life history phenotype (bank), raised in another's habitat (wood debris), resulted in significant response to selection in several traits (towards the phenotype typical of the transplant habitat); this was associated with an increase in absolute fitness. The habitat type included in the selection experiment (wood debris) exists in the estuary as a result of the perturbation of the environment typical of the transplanted life history phenotype (bank).

A genotype analysis (recombinant DNA techniques) demonstrated that wood debris populations were not more genetically similar than populations at large. This indicated that the observed variation in life history traits was the result of selection of independent genotypes. In two instances, the analysis indicated the wood debris life history phenotype evolved from a bank ancestor within the same estuary. This corroborated the results of the selection experiment and the information on change in habitat type due to natural perturbation.

All populations could be crossed reciprocally and produce viable F_1s and F_2s. The genotype analysis indicated that although all *Fucus* populations were distinct from bank and wood debris they were not as distinct as the only other species in this genus.

DEDICATED

to my

Mother and Father

ACKNOWLEDGEMENTS

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Maureen Connelly was the best friend and companion anyone could ask for, at a time when I needed friendship the most. The unfaltering love and support of my family: Bruce, Lynne and Shelley Stanhope, provided much comfort, security and strength throughout this entire period.

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GENERAL INTRODUCTION

The pioneering works of Cole (1954) and MacArthur and Wilson (1967) established the basis for development of a theory of life history evolution. MacArthur and Wilson's book stimulated much of the theoretical and empirical work in this field over the last 20 years. They clearly recognized the importance of genetics to an understanding of life history evolution and called for heritability estimates of life table parameters. Life histories provide ideal subject matter for the study of the evolutionary interplay between genetics and ecology. This is primarily because an organism's fitness is ultimately a consequence of its schedule of births and death. It is for this reason that life history theory is an important topic in evolutionary biology.

The ecological theory underlying life history evolution has received considerable attention (eg. Stearns, 1976; Roughgarden 1979; Bell 1980). Genetic studies of life history variation have until relatively recently lagged somewhat behind; however, over the last few years there have been numerous studies reporting heritable inter - and intra - population variation in life history traits (eg. Dingle et al. 1982; Allan 1984; Reznick 1982; Reznick and Bryga 1987; Grosberg 1988). Such studies place the measurements of genetic variation in an ecological context with varying degrees of emphasis and success. Measurement of natural selection of life history traits, by definition, indicates the focus is on genetic variation in an ecological context.

The selection / adaptationist program has come under much criticism over the last 15 years, particularly because some adaptationists have gone too far in attempting to explain the significance of particular characteristics (see Gould and

Lewontin 1979). Many investigators comment on selection and the adaptive significance of variation in a character, without verifying the genetic basis. More rigorous attention to the fundamentals of selection will help alleviate much of this criticism. Endler (1986) suggests that the best way to demonstrate selection is through the use of a combination of different methods. He lists over 250 studies of animals which demonstrate natural selection. Of these, fewer than ten deal specifically with life history traits.

Recombinant DNA techniques are not yet widely used in ecological studies. One of the distinct advantages of such methods is that they provide great precision in the identification of relationships between individuals or groups. A good example in this regard is the work of Quinn and White (1987), who identified a series of genomic DNA polymorphisms for use in studying gene flow, as well as maternity and paternity in colonies of the lesser snow goose. Recombinant DNA techniques are an important part of the work presented in this thesis. The precise information on the relative genetic relationships between groups that these techniques provide, forms an integral component in the following demonstration of natural selection.

In this thesis, I demonstrate strong diversifying natural selection of life history traits in an estuarine amphipod through an interdisciplinary approach which integrates ecology, quantitative genetics and recombinant DNA technology. The species chosen in this study was based on an earlier study of population dynamics (Stanhope and Levings 1985), in which obvious differences in voltinism between different habitats within the same estuary, were evident. I show that the selection is substrate driven (with an indication that the more precise factor is food availability), that individuals are adapted to their respective habitats and this adaptation is at least partly due to their array of life history traits. Analysis of genotype, through the use of

recombinant DNA techniques, indicates the relative genetic relationships between all populations and provides evidence that the members (populations) of one life history type evolved through independent selection events of different ancestors. As such, I feel it is an example of intraspecific parallelism, or convergence in life history traits.

CHAPTER I

SELECTION CONDITIONS, TEST, AND EXPERIMENT

INTRODUCTION

Over the last two decades life history studies have received intensive theoretical and empirical treatment. Stearns (1976) organized many of the earlier ideas on the evolution of life histories, including r and k selection (MacArthur and Wilson 1967; Pianka 1970), as well as several models suggesting mortality on various age classes as the important selective agent (Istock 1967; Murphy 1968; Emlen 1970; Schaffer 1974). He defined life history tactic as "a set of coadapted traits designed by natural selection to solve particular ecological problems. A complex adaptation". In an updated view of life history evolution Stearns (1980) raised the concern that there was little evidence to support local life history adaptation due to selection and suggested that physiological and developmental constraints may prevent the detection of life history tactics at an intraspecific level. Part of the problem in recognizing a life history tactic lies in identifying the ecological problem and deciding if an array of life history traits represents a solution to that problem. Questions remain as to whether life history traits can be driven to a number of local adaptive peaks and if so, at what rate.

The process of natural selection has three conditions: (1) variation; (2) fitness differences associated with the variants; (3) inheritance. The presence of these three conditions within a population is necessary and sufficient for natural selection to proceed. If natural selection can be used as an explanation for observed differences between populations then the three conditions must still hold (provided the environments have remained relatively constant), now however, on an inter - population basis, because they were necessary originally for the differences to arise. The process of natural selection should be detectable through the various means

summarized by Endler (1986). One of the most common and oldest methods is correlating traits with environmental factors. If some characteristic(s) of a habitat is a selective factor for a particular life history trait(s) then one should find a similar trait (or array of traits) in similar environment types. Traits not subject to selection by the factors present in the particular environment will vary independently. Endler points out that this method is indirect and does not demonstrate, but suggests selection. Another means of detecting selection is through the perturbation of natural populations. If traits are selected their distributions will change from what they were, subsequent to perturbation. Endler suggests that the best approach for conclusive demonstration of natural selection is usually a combination of methods.

Since Stearns (1980) expressed his concern that there was little evidence to support intraspecific life history evolution a number of studies have reported genetically based intraspecific variation in life history traits with combinations of traits suggestive of life history tactics (see for eg. Reznick and Endler 1982; Allan 1984; Berven and Gill 1983; Wyngaard 1986a,b). More convincing evidence of the possibility for intraspecific fine tuning of life history traits and combinations of traits has come from experimental selection studies (see for eg. Doyle and Hunte 1981a,b; Barclay and Gregory 1982; Bergmans 1984; Reznick and Bryga 1987). These studies have the benefit of addressing questions regarding selective agents and thus the nature of the ecological problem(s) and its solution(s).

This chapter documents microgeographic variation in life history traits of an estuarine amphipod (*Eogammarus confervicolus)* in three habitat types within the same estuary and shows through reciprocal transplant experiments and interpopulation crosses that this variation has a genetic basis. I then show that an

amphipod's fitness is greatest in its native habitat. The presence of the three selection conditions on an interpopulation basis suggests the observed variation in life history traits is the result of natural selection. Additional evidence for selection came from correlating traits with environment type; the habitat types are easily replicated on a regional scale in British Columbia. Since one of the habitat types exists as a result of the perturbation of one of the other habitats this provides an additional test of selection. Knowledge of the approximate timing of this disruption provided a time frame for estimating divergence in life history traits. A selection experiment, designed to simulate the perturbation in estuarine habitat, then presents information on targets and agents of selection and evidence for life history adaptation.

MATERIALS AND METHODS

Species Description

Eogammarus confervicolus is the most abundant and widely distributed gammarid amphipod on the North American Pacific coast (Bousfield 1979). It occurs intertidally in estuaries amongst sedge, under various species of algae, woody debris or stones and along protected shores that have brackish waters. Its salinity tolerance is between 5-25‰; optimum is between 5-10‰ (Sharp 1980). It is a major food organism for juvenile salmonids as well as comprising a portion of the diet in herring, sculpin and flounder (Goodman and Vroom 1972; Levy and Levings 1978; Levy et al. 1982). Development is direct and takes place in the female's marsupium. Gammarid amphipods graze on epiphytic fungi, bacteria, diatoms and various macrophytes (Hargrave 1970; Kostalos and Seymour 1976; Sutcliffe et al. 1981).

The Habitats

Three habitat types were included in this investigation: woody debris, a particular type of *Fucus distichus* community and embankments along the perimeter of *Carex lyngbyei* marshes. Logs have been stored in estuaries in British Columbia (and throughout the northeast Pacific) for 75-100 years. In areas where log booms ground at low tide the result is a mud flat devoid of macrophytes with accumulations of bark fragments in depressions along the surface. *Eogammarus confervicolus* was found amongst such wood debris. Bark pieces ranged in size from very fine particles less than 250 um to pieces several metres long and were layered between a few centimetres and 25 cm deep. Such areas are commonly about 2.0 m above mean low water.

The second principal habitat type was a mixture of two brown algae: *Fucus distichus* and *Pelvetia fastigiata* (hereinafter termed *Fucus*) overlying soft dark black mud smelling of hydrogen sulphide. The amphipods were found within the algal mixture and at the mud surface. This algal community usually occurs as relatively small patches (rarely exceeding 1 ha) in estuaries or quiet, brackish water bays at about 3.5 m above mean low water.

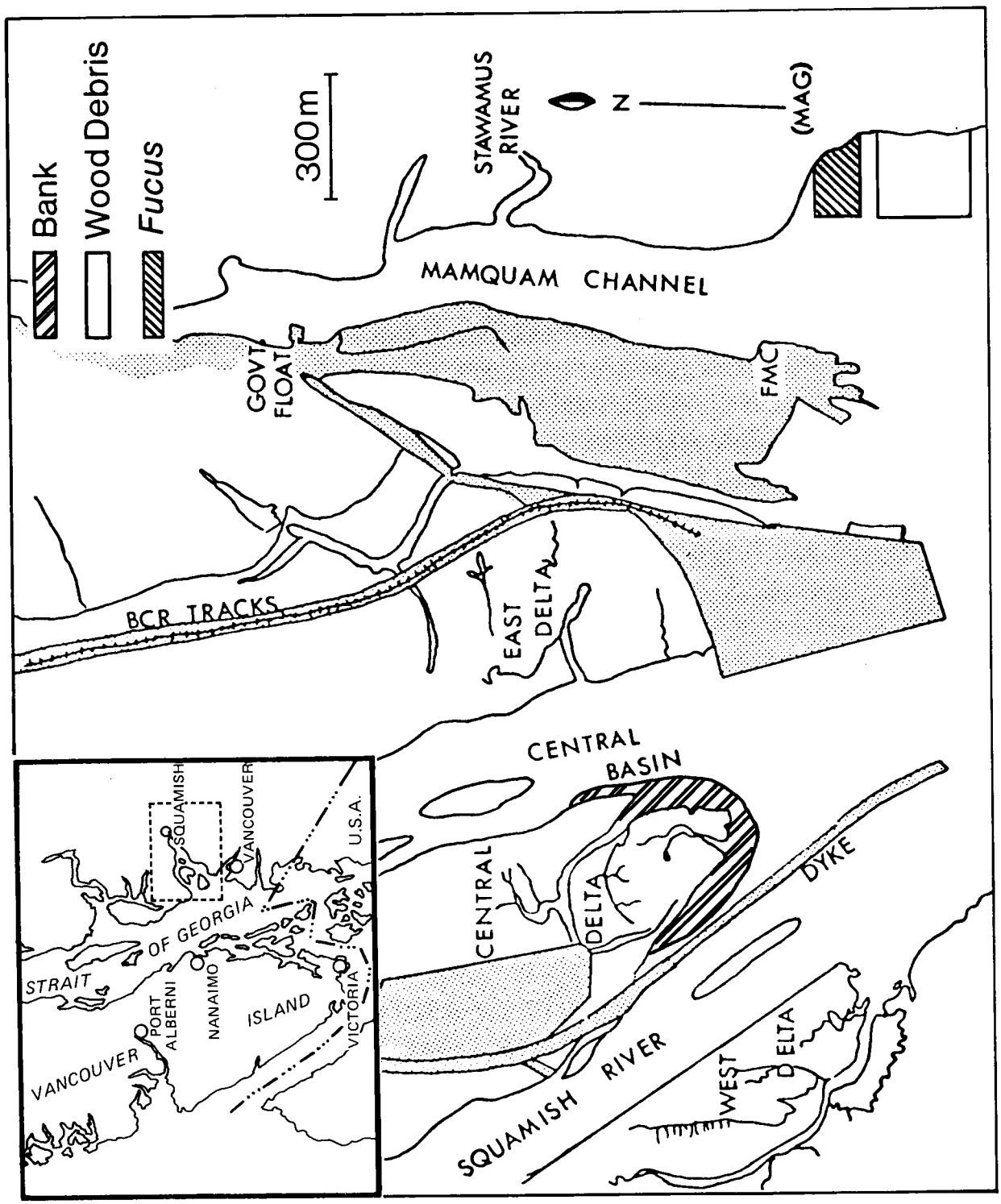
Many estuaries of the northeast Pacific possess deltas covered predominately with *Carex lyngbyei*. The perimeter of such a marsh is an embankment over which hangs a mat composed of sand, clay and *C. lyngbyei* rhizomes (hereinafter termed bank) at about 2.0 m above mean low water.

One of each of these habitat types exists in the Squamish River estuary, B.C. (Fig. 1). Members of populations from these three habitat types formed the basis for study of life history variation in this amphipod species. Wood debris and *Fucus* habitats were separated by only a few hundred metres of mud flat whereas the distance between bank and wood debris habitats was approximately 2 km, with a variety of physical obstructions in the middle of the estuary.

Cultures

Most of the life history measurements necessitated keeping animals in the laboratory. Two types of apparatus were established for this purpose. Larger cultures were maintained in 50 l aquaria at the Department of Fisheries and Oceans, West Vancouver Laboratory, or in 30 l aquaria at S.F.U.. Substrate from the three habitat types was brought from the Squamish estuary and established in these aquaria. Tanks simulating a wood debris habitat contained wood chips overlying mud; those simulating the bank habitat contained a piece of the rhizome mat and the *Fucus*

Fig. 1. Squamish River estuary, at the north eastern end of Howe Sound (indicated as dashed outline in the figure inset), showing the major physical features and locations of the three original sites. The bank population was located along the perimeter of the Central Delta; the wood debris and *Fucus* populations were at the mouth of the Mamquam Channel. Stippled areas indicate landfills.


Bank
Wood Debris
Fucus
300m
STAWAMUS RIVER
N
(MAG)
MAMQUAM CHANNEL
GOVT FLOAT
FMC
BCR TRACKS
EAST DELTA
CENTRAL BASIN
CENTRAL DELTA
DYKE
WEST DELTA
SQUAMISH RIVER
SQUAMISH
VANCOUVER
U.S.A.
STRAIT OF GEORGIA
PORT ALBERNI
NANAIMO
ISLAND
VICTORIA
VANCOUVER

simulation was a mixture of *F. distichus* and *P. fastigiata*. Water was kept at 10 - 12°C and salinity between 8 - 14‰, depending on the season. Water was well aerated and changed at least twice a week (by controlling a drain in each of the 50 l aquaria that was surrounded by fine-mesh screen to prevent the loss of either substrate or animals) at which time the substrate was left exposed for several hours, to simulate the occassional low tide (the purpose here was primarily to keep the algae fresh). Photoperiod was maintained approximately natural. Substrate was replaced regularly with fresh material from the estuary.

For the purposes of most life history measurements animals were kept individually, as mating pairs or in small numbers in 2 l or 750 ml vessels, where they could be monitored every day. Conditions were similar to those of the larger cultures except the water and substrate were changed more frequently (every few days).

Selection Conditions

1. Life History Variation

Fourteen life history traits were measured in each of the three sampling sites within the Squamish estuary (Fig. 1): number of broods per female (BRD), development time (DEV), interclutch interval (INTCLU), primary sex ratio (SEXR), size at reproductive maturity (RESZ), life span (LFSP), brood mortality of 11.0 mm (±0.5 mm) females (BRDMRT), maximum size attained by females (MXSZF), age at reproductive maturity (AGERP), size of eggs (EGSZ), weight of newly released juveniles (JUVWT), mortality prior to reproductive size (JUVMRT), number of eggs per 11.0 mm (±0.5 mm) female (EGGS) and maximum size attained by males (MXSZM). A list of all the acronyms used in this thesis appears in Appendix I. All

measurements except fecundity and size of eggs, necessitated rearing animals in the laboratory. Fecundity and egg size were the only two characteristics that were routinely measured from the same individuals. Animals were reared on the substrate from which they were collected (the reciprocal transplant experiment discussed later in this chapter indicates the difference it makes when amphipods are raised on alternate substrates). Development time was taken as the time between release of the female by the male (termination of amplexus) and the first appearance of juveniles (first brood). Interclutch interval was the time between release of juveniles till the termination of amplexus at the next mating (interval between first and second brood). I chose this measure of "interclutch" interval to avoid overlap with development time; more precisely, this "interclutch" interval is a measure of the time between release of juveniles and the next fertilization. Reproductive maturity was taken as the first appearance in amplexus. Estimates of life span are for females and refer to age at death. JUVMRT was the percentage of a female's first brood that did not make it to reproductive maturity. Brood mortality was computed as the difference (expressed as a percentage) between the number of juveniles released and the average fecundity of 11.0 mm (+0.5mm) females. Ten eggs per female were measured for egg size estimates. All the newly released progeny of a female's first brood were counted and collectively weighed (dry weight) to obtain individual JUVWT estimates. Fecundity and egg size of animals raised (from juveniles) in their respective laboratory substrates were not significantly different from those of females collected in the estuary and thus, I was confident of both the accuracy of my brood mortality estimate and the nature of the habitat simulations. Sex ratio was measured as the percentage of a female's offspring that are male at reproductive maturity.

2. Fitness

My definitions of fitness are after those of Endler (1986); absolute fitness was measured as the absolute lifetime contribution to the breeding population (number of individuals produced per female that reach reproductive size); relative fitness was measured as the average contribution to the breeding population by the members of a particular habitat type relative to the average contribution of members of other habitat types. Measurements of fitness were made for each of the three Squamish populations, in the laboratory simulated habitat types. A population's mean absolute fitness was determined in their native substrate (ie. the substrate from which they arose) and in each of the alternative laboratory simulated habitats. To be more specific, juvenile amphipods were field collected from each of the estuarine habitats and raised in each of the laboratory habitats (for example, field collected amphipods from the bank habitat were raised in *Fucus*, wood debris and bank substrate) and their absolute fitness was determined in each of those situations. I was interested in measuring whether fitness differences were present on an interpopulation basis. A measure of fitness differences then, was the ratio of a population's mean absolute fitness in its native substrate to that of members of the other two habitat types raised on that substrate. For example, the fitness of bank amphipods relative to wood debris was taken as the ratio of the mean absolute fitness of amphipods collected in the estuarine bank habitat and raised in bank substrate to the mean absolute fitness of amphipods collected from the estuarine wood debris habitat and raised in bank substrate. A ratio of the mean absolute fitness of bank animals in bank substrate to the mean absolute fitness of wood debris animals in bank substrate that was significantly greater than 1 was taken as evidence for fitness differences associated with the variants (where variants are considered the life

history phenotypes typical of each habitat type) and as evidence that selection would act in favour of the life history resident, against (in this particular example) a wood debris life history immigrant. This means of comparing fitnesses was particularly relevant in this situation, since the populations concerned are within the same estuary and in the case of wood debris and *Fucus*, only separated by approximately 300 m of intertidal mud flat.

A different approach was needed to determine relative fitness for the *Fucus* population in its native substrate. Life span and timing of reproduction of individuals from the three populations was such that two generations were produced annually in bank and wood debris while the *Fucus* population was univoltine (Stanhope and Levings 1985). A measure of relative fitness between *Fucus* and the other two populations, must then, include the reproductive output of both generations in bank and wood debris vs the single generation in *Fucus*. A period of 540 days was sufficient time for a juvenile amphipod from the *Fucus* population to grow to maturity, reproduce and for the progeny to reach approximate reproductive size. Bank and wood debris amphipods reproduced at about 180 days, the progeny grew to reproductive maturity at approximately 370 days and the second generation reached approximate reproductive size at 540 days. It was not surprising then, to find that bank and wood debris animals raised in *Fucus* substrate had a reproductive success approximately five times greater than *Fucus* animals in their native substrate after 540 days (total at 540 days divided by one half the initial number of inoculants; mean of two aquaria). This indicated however, that relative fitness in *Fucus* substrate favoured bank and wood debris amphipods and raised the question of why they had not overtaken the *Fucus* environment, especially since the Squamish wood debris and *Fucus* populations are only separated by a few hundred metres of intertidal

mudflat. This situation prompted me to measure the reproductive success of bank and *Fucus* animals, when raised together in *Fucus* substrate and similarly the reproductive success of wood debris and *Fucus* animals when raised together in *Fucus* substrate. Since there is considerable overlap in morphological characteristics between the groups, such measurements are not diagnostic; therefore, I chose to characterize the relative composition after 540 days using recombinant DNA techniques. Such methodology is ideally suited for this purpose because of the great precision to which one can fingerprint the groups. These techniques provided the necessary markers to use in competitive ability experiments between *Fucus* amphipods and the members of the bank and wood debris populations.

Equal numbers of juvenile amphipods from two populations were inoculated into a series of aquaria containing *Fucus* substrate. At 540 days all animals from an aquarium containing an interpopulation mixture of *Fucus* and bank, or Fucus and wood debris, were collected, counted, remixed, two sets of 100 and a subsample of 10 removed for DNA extraction. Five aquaria were established of each interpopulation mixture. The DNA methods employed are standard ones and are described in many recombinant DNA laboratory manuals (eg. Maniatis et al. 1982; Berger and Kimmel 1987). I provide a brief summary here; a more detailed description appears in Chapter 2. DNA was extracted by grinding fresh amphipods in a solution containing diethyl pyrocarbonate, purified on CsCl gradients, cut with restriction endonucleases and run out on agarose gels. DNA in the gels was then transferred to nitrocellulose through the procedure known as Southern blotting. A small library of unique fragments were cloned (randomly) from the genome of the bank, *Fucus* and wood debris animals, labelled with ^{32}P through the procedure known as nick translation and used as probes in hybridizing to the nitrocellulose

filters. The library had been established for the purposes of a detailed examination of genetic variability among these populations. A probe was chosen which in combination with a particular restriction endonuclease, both simply and uniquely distinguished *Fucus* amphipods from bank and wood debris (a single band for each population). This probe was used to hybridize to DNA isolated from the pooled sets (100 amphipods in each of two sets) and to that isolated from each of the 10 amphipods removed as a subsample. In this manner the relative composition of each aquarium at 540 days could be determined. If both genotypes were present at 540 days then the radioactive intensity of bands involving pooled DNA sets (measured by cutting out bands on hybridized filters, dissolving the nitrocellulose and placing in a scintillation counter) would reflect the relative composition in the aquaria. Differences in the intensity of the respective bands were an indication of fitness differences between the members of population pairs when raised together on *Fucus* substrate. Since I am concerned with interpopulation variation in life history traits, this is synonomous with fitness differences asssociated with the variants (the variants being the life history types typical of each population). Analysis of the 10 individuals provided an indication of the degree of cross breeding between the life history types, when raised together in the same aquaria (F_1 hybrids should possess both bands). If hybrids existed at all, they were expected to be a small percentage, since the overlap in reproductive maturity between *Fucus* and the other two life history types was only a twenty or thirty day period and differences in size at this time were considerable. From a knowledge of the initial number and the total after 540 days, concomitant with the relative composition (using the DNA data), I calculated an approximate reproductive success for bank and *Fucus* amphipods when raised together on *Fucus* substrate and a similar estimate for wood debris and *Fucus*

amphipods when raised together on *Fucus* substrate. Relative fitness then, of *Fucus* amphipods in their native substrate, was the ratio of these approximate values of 540 day reproductive success.

3. Inheritance

Reciprocal Transplant Experiments

Reciprocal transplant experiments were used to assess the relative contribution of genetic and environmental factors. A genetic basis to the variation between populations was demonstrated when there was essentially no change of the mean value of a trait after transplant, or the between habitat changes were very small. Only those traits that had proven variable between the three Squamish populations were included in this analysis. Transplants were maintained for three generations. Maternal effects due to differences in the nutritional state of mothers from the three habitat types would be expected to affect the first generation of transplant. If there was no change in the mean of a trait between generations then maternal effects were assumed not to be important (the results of the interpopulation crosses address the issue of maternal effects more directly). Juvenile amphipods were collected from the estuary in each of the respective habitats and established in every combination of transplantation involving the three substrates, including animals raised in their own substrate type as controls. These transplants were performed in the simulated laboratory habitats (50 l aquaria). For example, juvenile amphipods from the estuarine *Fucus* population were collected and established in the laboratory *Fucus*, bank and wood debris substrates. Juvenile amphipods from the other two locations were also raised in each of these three substrates. Each of these aquarium cultures were maintained for three generations. Animals were sampled

from these aquaria, at each generation and placed in the 2 l and 750 ml vessels from which life history measurements were made. Generation one life history measurements were made on the field collected amphipods when they reached reproductive maturity; their progeny were designated generation two. Age at reproductive maturity and life span were two life history characteristics which required knowledge of when individuals were born. In this case adults were collected from the three field situations and the generation one measurements were made on the progeny of the field caught adults. Generation one measurements of mortality prior to reproductive size required collecting animals that were just under average reproductive size and allowing them to reach reproductive maturity in the laboratory. The first brood progeny of these individuals were then used to make the JUVMRT measurements.

A three factor analysis of variance was used to analyze this transplant data. The three factors were population (the field collected origin of the amphipods), habitat (the laboratory substrate in which they were raised) and generation. The population effect can be considered synonomous with genotype, habitat with environment and the population - habitat interaction, synonomous with genotype - environment interaction. There were three populations, the members of which were raised in three habitats for three generations. Analysis of variance was performed for each life history trait. A larger F statistic for the population effect than for the habitat effect indicated the variation depended more on the estuarine source of the amphipods (ie. which population they arose from) than on where they were raised (ie. which laboratory substrate). If such a result was also associated with a non - significant generation effect then I concluded a genetic basis to the trait variation with some effect of environment. A rigidly fixed trait was one in which there was a

highly significant population effect, a non - significant habitat effect and a non - significant generation effect. A trait whcih was more affected by environment than genetics, exhibited a larger F statistic for the habitat effect than for the population effect. Significant population - habitat interactions indicated that the degree of environmental induction differed between populations. A multiple range test on this interaction data provided an indication of the degree to which each population was affected by transplantation and which substrate was most effective in causing this induction. The benefit of this test then, lies in that it not only provides genetic evidence but also provides information on the degree to which traits are modulated and which substrate is most effective in causing this modulation.

Interpopulation Crosses

Reciprocal, interpopulation crosses were performed between the three groups from Squamish. The crosses between wood debris and bank were performed in bank substrate (chosen because there was much less effect on transplanting wood debris animals into bank substrate than for the reverse situation). The life history traits measured in the F_1 were brood number, development time, life span, egg size, fecundity and interclutch interval. The cross involving wood debris and *Fucus* amphipods was performed in *Fucus* substrate; that between *Fucus* and bank, in bank substrate. The life history traits measured in crosses involving *Fucus* amphipods were the same as in the cross between wood debris and bank with the addition of age at reproductive maturity. All reciprocal interpopulation crosses were established using approximately 50 pairs. Females were held separately, for a few weeks prior to crossing, to assure the release of any offspring resulting from an earlier fertilization and to verify that there was no parthenogenesis. Thirty pairs were removed from the

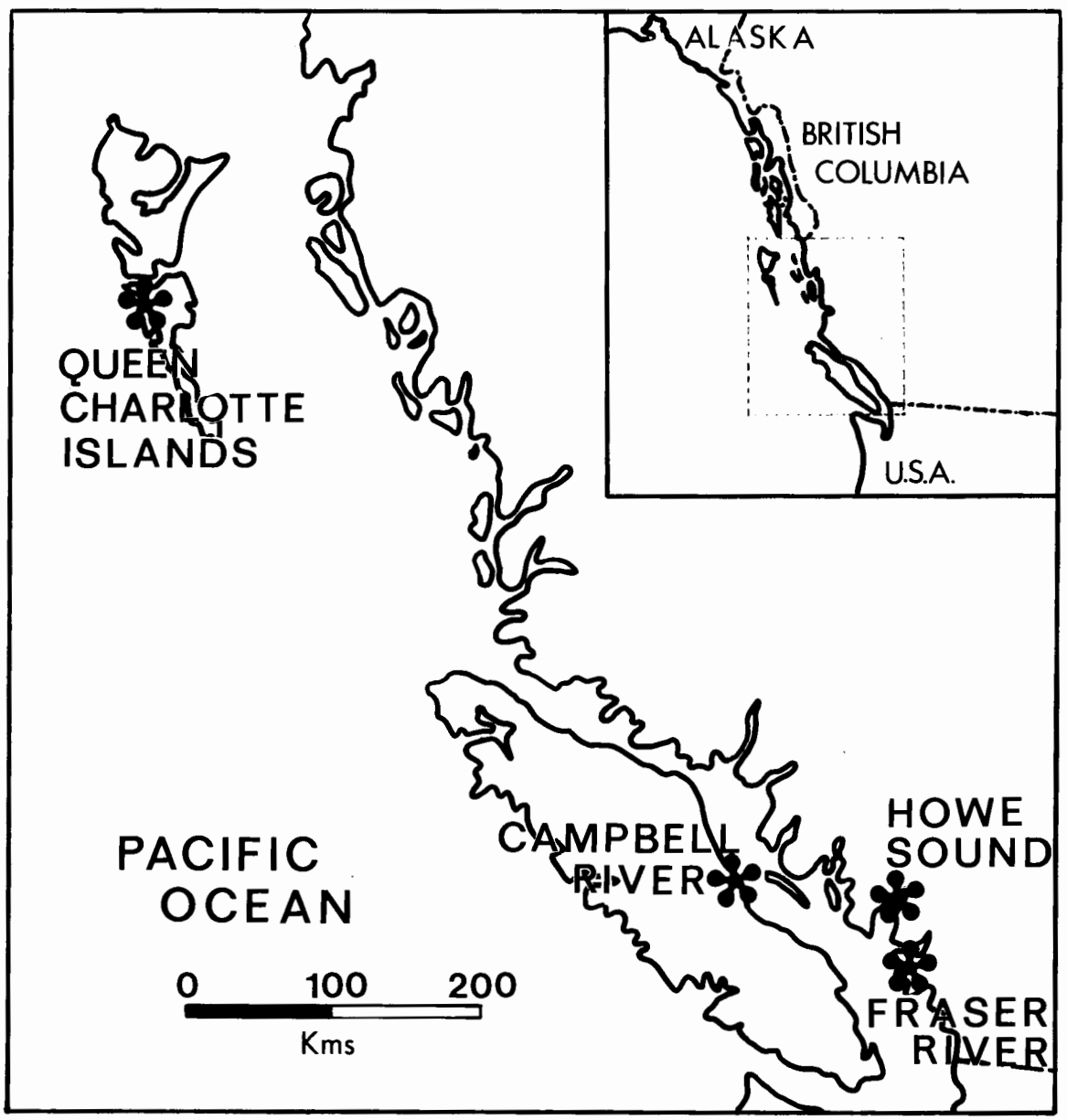
F_1 of each interpopulation cross and used to make the life history measurements, ie. since most of the measurements are reproductive in nature, F_1 data required mating, and thus hybrid females were mated to hybrid males of the same cross.

If most of the genetic variance underlying quantitative traits is additive, then interpopulation hybrids will be intermediate. As non-additive components (chiefly dominance) increase in importance, population hybrids will deviate from intermediacy. A measure representing the degree to which F_1 hybrids differ from parental phenotypes was presented by Wright (1978): F_1 mean minus the lower parental mean / the upper minus the lower parental mean. The parental means were taken from the results of the transplant experiment. An intermediate hybrid phenotype is represented by a value of 0.5. I use this measure as a means of assessing dominance in interpopulation crosses involving these three populations.

Selection Test

Correlation of life history traits with habitat type provided further evidence for selection (Method I described by Endler 1986). If some characteristic(s) of a habitat is a selective factor for a particular life history trait (or array of traits) then one should find similar traits in similar environment types. The three habitat types are easily replicated on a regional scale in British Columbia. Six additional populations were included as part of the selection test: one of each habitat type in an estuary in the Queen Charlotte Islands, a *Fucus* population in Howe Sound (southern B.C. coast), about 4 km south of Squamish (ie. the Squamish estuary is at the north end of Howe Sound), a log storage area in the Campbell River estuary (Vancouver Island) and a bank population from the Fraser River estuary (Fig. 2). The wood debris habitat in the Queen Charlottes was not the result of log storage activities, but

Fig. 2. West coast of British Columbia showing the location of the additional populations included as part of the selection test.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U.S.A.
QUEEN
CHARLOTTE
ISLANDS
PACIFIC
OCEAN
CAMPBELL
RIVER
HOWE
SOUND
FRASER
RIVER
0
100
200
Kms

apparently the consequence of natural deposition of wood debris from upstream. The correlation analysis involved all nine locations and included those life history traits that had proven variable in the Squamish populations and in which the variability had more of a genetic than an environmental basis.

Transplants involving the six additional populations were also performed to verify that conclusions regarding environmental and/or genetic components of life history variation in the Squamish populations were applicable to the others. Transplants in this case involved two sets of three populations: the Queen Charlotte populations and the lower mainland and Vancouver Island populations. There was a population represented from each of the three habitat types in each of these two sets. Amphipods from the populations comprising each of these sets were established in every combination of transplant involving the three principal substrates, in the same manner as described for the Squamish populations. The only difference between these transplants and those described earlier, is that in this case they were only maintained for a single generation. The two sets (three populations each) were analyzed separately using analysis of variance in the same manner as for the Squamish populations.

Interpopulation crosses involving the wood debris populations from Campbell river and the Queen Charlottes were also performed. Each of these populations were crossed reciprocally with the bank population from Squamish, in bank substrate.

Data Analysis

Stepwise multiple discriminant analysis (MDA) was used to summarize the extent of overall life history variation in the Squamish estuary and as a means of illustrating the degree to which overall life history phenotypes were correlated with habitat type. The MDA included both discriminant functions and Mahalanobis generalized distance (D^2 statistic). A discriminant function is a linear equation that best discriminates between groups, based on multiple characteristics. Generalized distance, which can be derived from the discriminant function, is a measure of distance between group means. Population centroids (multivariate population means) and multivariate means for individuals, were plotted in two dimensional space. The hypothesis was that habitat specific selection for particular life history phenotypes should result in the centroids of populations from similar environment types lying closer in discriminant space than those of disparate habitat types. The equality of multivariate population means was tested statistically by transforming generalized distances to an F statistic (Morrison 1967).

MDA is useful for a comparison of the overall life history phenotype but does not provide much information about the extent or nature of variation in individual traits. For summarizing the variation in individual traits I chose two statistical methods: a quick cluster analysis (SPSS Inc., 1986) and nested single factor analysis of variance. The quick cluster algorithm produced clusters by assigning cases to the nearest cluster center (measured by squared Euclidean distance). I set the cluster centers as the mean of a trait from each of the three habitat types. By coding each of the nine populations into one of the three habitat types I could then compare the cluster membership with habitat classification for each trait. Crosstabulation of the data indicated the proportion of animals sampled from a

particular environment type that fell into the three habitat classifications. Chi - square was used to analyze this contingency table and an uncertainty coefficient was computed as a measure of the ability to predict the value of a particular life history trait in each of the respective habitat types (or conversely, the habitat type in which a particular value of some life history trait would most likely be found). An uncertainty coefficient of 1.0, concomitant with a significant chi - square, would indicate that all animals measured were perfectly associated with the habitat type from which they arose (A clarification is necessary here: uncertainty coefficients will be high when observations are distributed evenly among categories. In this particular example this would result when measurements were distributed randomly amongst the three habitat types, which would result in an insignificant chi - square, or when there was a strong association between habitat type and source). Selection was suggested from the nested anova when there was a significant habitat effect (populations were nested within habitat type). Results of the crosstabulation data and nested anova were considered in concert with the results of the transplant experiment to decide which traits were selected.

Sample size was 30 for the MDA of life history traits and the reciprocal transplant of Squamish populations; sample size was 20 for transplantation of the additional six populations. Details of the transplant analysis have already been provided (see section on inheritance).

Selection Experiment

The selection experiment was designed to test whether wood debris subtrate *per se* was a selective agent for any of the measured life history characters. Bank amphipods from the Squamish estuary were raised in wood debris substrate for 8

generations (a continuation of the transplant experiment). The combination of bank animals and wood debris substrate was chosen because I had good reason to believe the wood debris population arose through the perturbation of a bank environment (see comments in discussion). Measurements of the life history traits included in the selection test as well as measurements of absolute fitness and clutch volume (fecundity X egg size) were made in generations 1-4,6 and 8 (measurements of egg size and fecundity were possible in generation 9 as well). Egg size and fecundity were measured in a control tank of bank animals in bank substrate and in a replicate transplant tank. These two characters were chosen in the controls because they were hypothesized to be those most affected by an alteration in food supply and because of their ease of measurement. Preliminary results of mine (Appendix II) and those of McKeag (1983) indicate wood chips support much reduced biomass of the microbes typically used as food by amphipods. Life history theory predicts that a resource scarce habitat should select for fewer, larger offspring (Stearns 1976). By using this combination of bank amphipods and wood debris substrate I am both simulating the direction of change due to natural perturbation and testing a specific aspect of life history theory. The response to selection was measured as the slope of a regression line fitted to the generation means of each trait (N=20).

Mating Success

To verify that all populations concerned in this study may satisfactorly be considered to comprise one species, I established a complete set of reciprocal crosses. Mating success was recorded as the percentage of crosses that produced offspring. A high percentage of fertile F_1 hybrids was taken as evidence that the populations comprised the same species. All crosses used unrelated individuals, a

single male with a single female and 30 pairs per cross. Females were held separately for three weeks prior to crossing, to avoid any posible confusion due to an earlier fertilization and to verify that there was no parthenogenesis. A three factor anova (location / habitat type / generation) and a separate anova for source of mother or father were used to detect any trends in mating abilities.

RESULTS

Life History Variation in Squamish Estuary

Multiple discriminant analyis separated three distinct life history phenotypes within the Squamish estuary (Fig. 3; Bartletts X^2=102.9; df=22; p<0.0001). Canonical discriminant function I explained 84% of the variance and function II, 16%. Generalized distances were much greater between *Fucus* and the other two populations than between bank and wood debris (480, 492 and 117 for *Fucus* / bank, *Fucus* / wood debris and wood debris / bank respectively). Of the fourteen life history traits, life span followed by fecundity were identified as the two variables exerting the greatest effect in discriminating the three groups. Age at reproductive maturity and life span were the traits most highly correlated to canonical discriminant function I (correlation coefficients of .80 and .25 respectively), indicating they were the most significant life history variables in discriminating between *Fucus* and the other two populations. Brood mortality and fecundity were the variables most highly correlated to discriminant function II (correlation coefficients of .56 and .38 respectively), indicating they were the most significant variables in distinguishing between wood debris and bank. All life history traits except sex ratio (which was 1:1 in all habitats) showed significant differences across the three Squamish habitat types (Table 1).

Fig. 3. Canonical plot from multiple discriminant analysis of Squamish populations. BK: bank; WD: wood debris; F: *Fucus*; SQ: Squamish. Each point represents the multivariate life history mean of an individual amphipod.

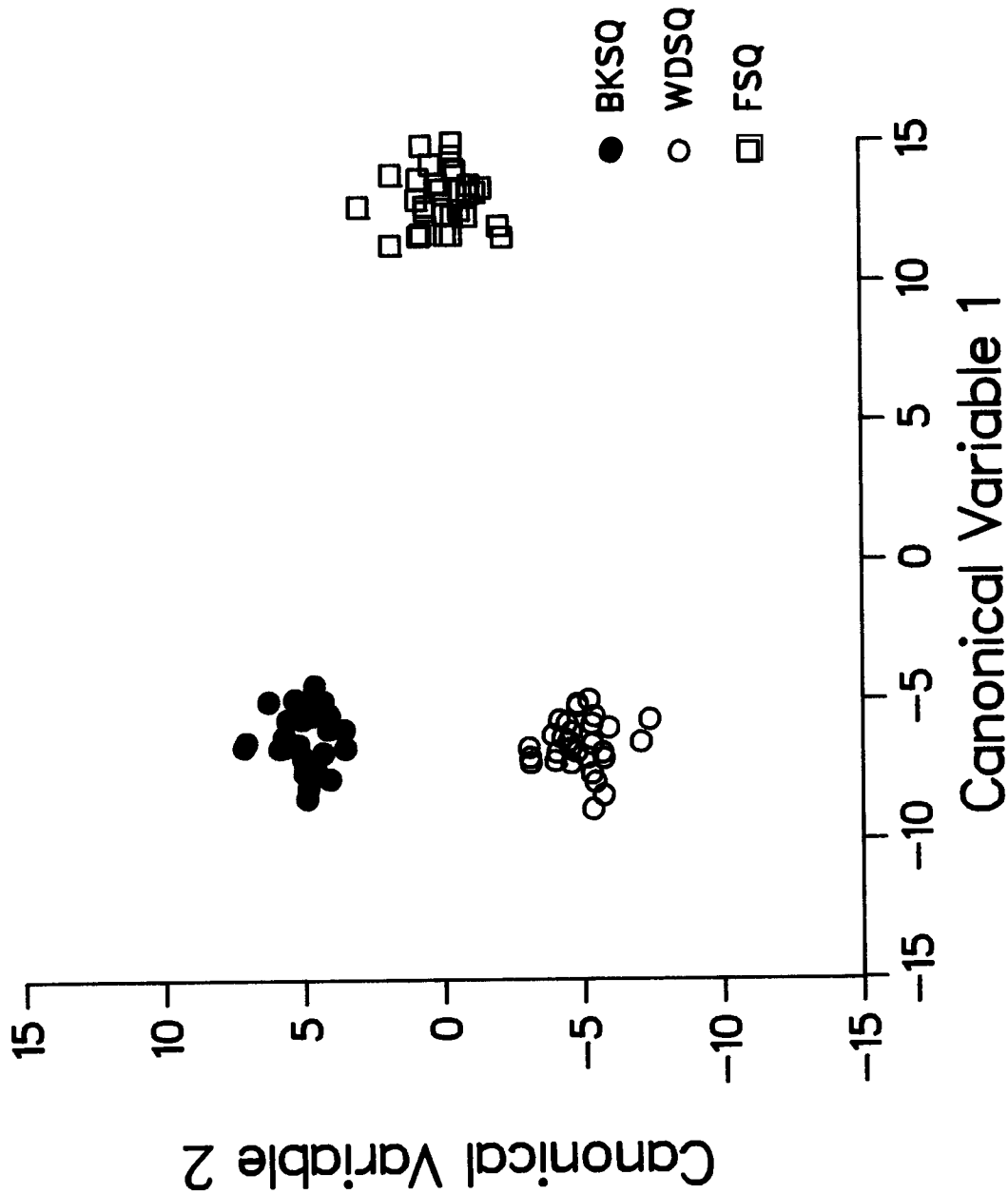

BKSQ
WDSQ
FSQ
Canonical Variable 1
Canonical Variable 2
15
10
5
0
−5
−10
−15
−15
−10
−5
0
5
10
15

Table 1. Results of analysis of variance (F statistics) on life history traits measured in the three Squamish populations. Actual values appear as part of selection test (Fig. 7). Trait abbreviations: BRD = brood number; DEV = development time; RESZ = size at reproductive maturity; LFSP = life span; BRDMRT = brood mortality; MSXZF = maximum size of females; AGERP = age at reproductive maturity; EGSZ = egg size; EGGS = fecundity; MXSZM = maximum size of males; INTCLU = interclutch interval; JUVWT = weight of newly released juveniles; JUVMRT = mortality prior to reproductive size. Significance levels: **** = $p < .05$; *** = $p < .01$; ** = $p < .001$; * = $p < .0001$.

Trait	F Statistic
BRD	28.07 *
DEV	65.48 *
RESZ	53.96 *
LFSP	262.30 *
BRDMRT	245.20 *
MXSZF	3.15 ****
AGERP	2259.00 *
EGSZ	9.23 **
EGGS	158.60 *
MXSZM	17.83 *
INTCLU	122.69 *
JUVMT	13.45 *
JUVMRT	5.15 ***

Fitness

An amphipod's absolute fitness was greatest in its native habitat type (Table 2). The absolute fitness of amphipods from *Fucus* was almost twice that of bank and wood debris. A significant drop in absolute fitness, upon transplantation into another habitat type, was evident in all cases. The greatest effect on absolute fitness of bank and *Fucus* amphipods was evident when they were raised in wood debris substrate.

An amphipod's relative fitness was also greatest in its native habitat. Compare for example, the mean absolute fitness of wood debris animals raised in wood debris substrate (56.7) to that of bank animals raised in wood debris substrate (23.3; ratio of 2.43) or the reverse situation: bank animals raised in bank substrate (54.0) vs wood debris animals in bank substrate (40.1; ratio of 1.35; $p<0.05$).

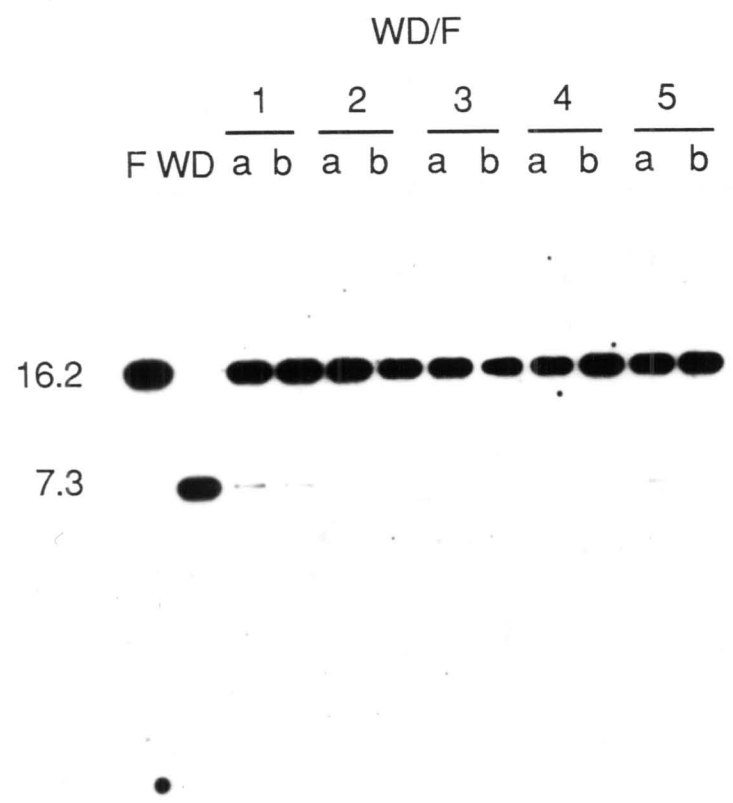
Due to the bivoltine nature of the bank and wood debris populations, their reproductive success in *Fucus* substrate over a 540 day period was much greater than *Fucus* animals (approximately five times greater, Table 2); however, when bank animals were raised with *Fucus* animals and when wood debris animals were raised with *Fucus* animals, the situation altered. Analysis of the pooled DNA sets (Fig. 4) indicated that after 540 days a mixture of bank and *Fucus* amphipods raised in *Fucus* substrate was approximately 30% bank animals; a wood debris and *Fucus* mixture, in *Fucus* substrate, resulted in a relative composition at 540 days of 17% wood debris animals. Relative fitness was now shifted in favour of the *Fucus* life history type. Despite the fact the bank and wood debris amphipods produce two generations vs the single generation of *Fucus* amphipods, they were at a fitness disadvantage relative to the *Fucus* resident, as long as the resident was present in approximately equal numbers at the start of the experiment. There was very little variation in estimated relative composition between the two pooled DNA sets taken from each

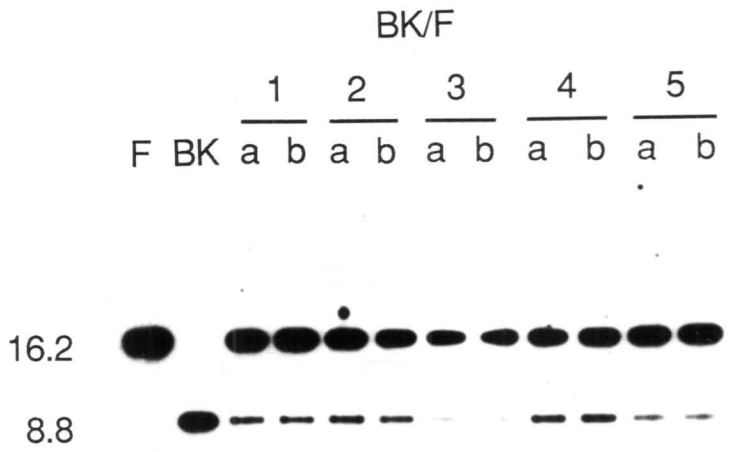
Table 2. Means and standard deviations (in brackets) of the fitness measures. RS refers to reproductive success; BK = bank, WD= wood debris; Bk/WD indicates bank amphipods were raised in wood debris substrate. The figures in the last column come from the results of the competitive ability experiment (Fig. 4) and refer to the reproductive success of bank and *Fucus* amphipods when raised together in *Fucus* substrate and that of wood debris and *Fucus* amphipods when raised together in *Fucus* substrate (reproductive success of *Fucus* is the "denominator" in each case). Compare these values to those in the second column, particularly those referring to the reproductive success of bank and wood debris amphipods when raised in *Fucus* substrate (Bk/*Fucus* and WD/*Fucus* respectively) without the presence of the *Fucus* competitor.

Situation	Absolute Fitness	RS/540 days	approximate RS/540 days -both phenotypes present
Fucus/*Fucus*	91.7(40.1)	127.4(65.2)	
Fucus/Bk	29.9(14.5)		
Fucus/WD	6.7(5.2)		
Bk/Bk	54.0(18.7)		
Bk/*Fucus*	40.9(19.5)	610.2(263.1)	36.0(28.1)/113.3(50.8)
Bk/WD	23.3(15.6)		
WD/WD	56.7(17.4)		
WD/*Fucus*	42.7(21.3)	682.5(287.9)	21.2(14.1)/124.1(73.6)
WD/Bk	40.1(22.2)		

Fig. 4. Autoradiograms of Hind III digests of pooled DNA sets probed with a radio - labeled fragment cloned from the bank population; two samples (a & b) taken from each of five tanks; relative intensities of the two bands in a particular lane indicates the relative composition in the tank; first two lanes for each situation are digests of DNA isolated from the two genotypes separately; sizes are given in kilobase pairs.

32b


WD/F
1 2 3 4 5
F WD a b a b a b a b a b
16.2
7.3

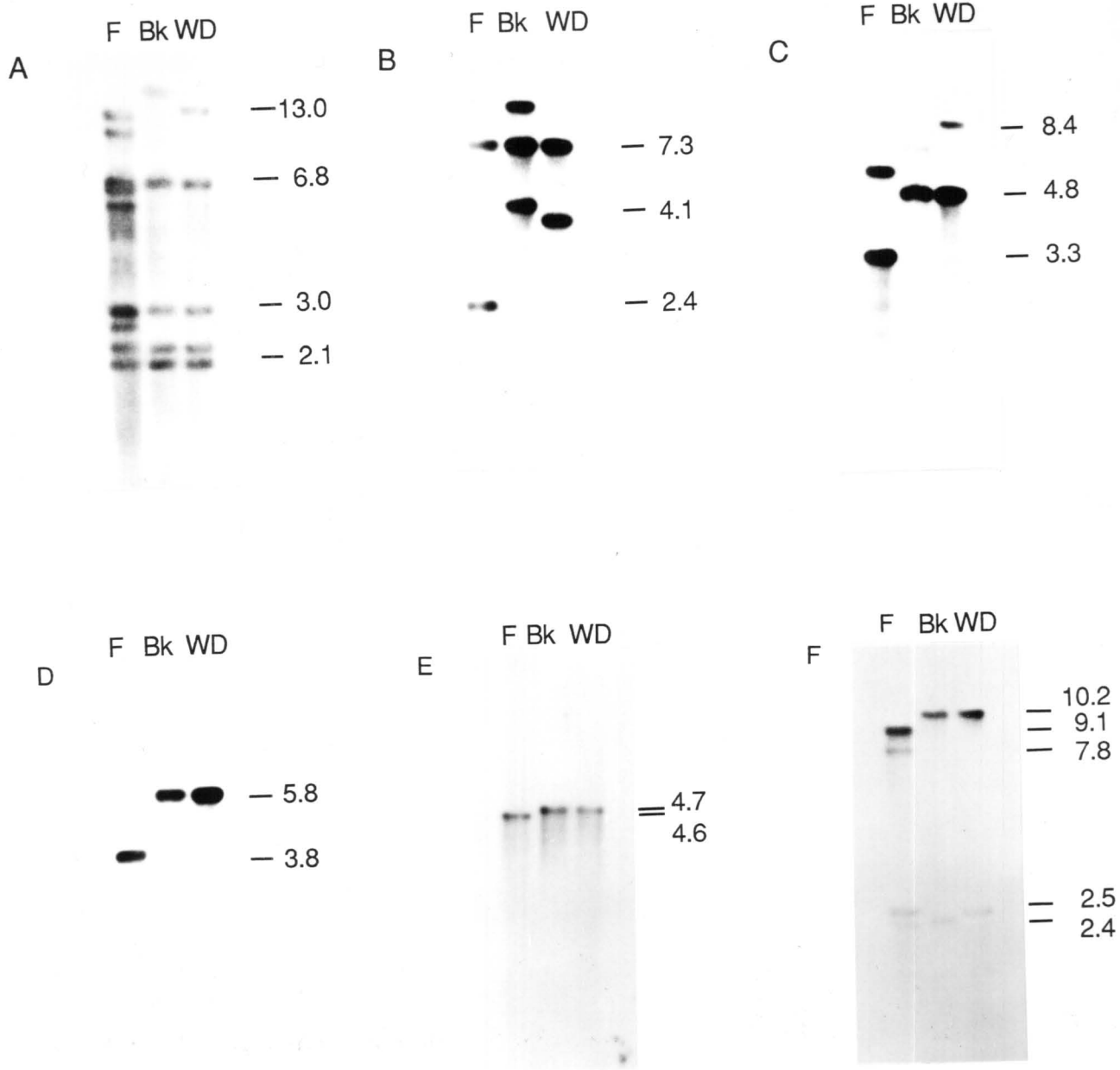

BK/F
1 2 3 4 5
F BK a b a b a b a b a b
16.2
8.8

tank (compare relative intensities of samples a & b from each tank in Fig. 4) and every tank indicated *Fucus* had the fitness advantage. There were no hybrids detected (both bands present in DNA isolated from single animals) in any of the tanks for either interpopulation mixture.

The RFLP (restriction fragment length polymorphism) used in this competitive ability experiment was not the only one identified between these three groups. Additional examples appear in Figure 5 and provide evidence that gene flow between the Squamish populations must be at a minimum. Significant gene flow between the groups would result in an absence of interpopulation polymorphisms. The polymorphisms indicated in Figure 5 appear to be population specific. The DNA in each of the lanes represented in this figure comes from pooled samples of 25 - 50 animals from the same population; faint representations of alternate genotypes within a given lane were never observed. Further evidence for the population specific nature of these RFLPs is presented and discussed in Chapter 2. It is this specificity that makes them useful markers in competitive ability experiments. A detailed examination of population genotypes using these techniques is the subject of Chapter 2.

Fig. 5. Autoradiograms showing examples of DNA RFLPs between the three Squamish populations. Each example involves the use of a different probe. Sizes are in kilobase pairs.

34b


A
F Bk WD
13.0
6.8
3.0
2.1
B
F Bk WD
7.3
4.1
2.4
C
F Bk WD
8.4
4.8
3.3
D
F Bk WD
5.8
3.8
E
F Bk WD
4.7
4.6
F
F Bk WD
10.2
9.1
7.8
2.5
2.4

Inheritance

Reciprocal Transplant Experiment

All traits showed a significant population effect that was, with the exception of maximum size of males and females, more pronounced than that of habitat type (Table 3). None of the life history traits exhibited a significant generation effect, suggesting maternal effects were not important in explaining the variation. Thus, the observed variation in each trait (except maximum size of males and females) was more due to genetics than environment. Some traits were more rigidly fixed than others. For example, development time and interclutch interval showed no effect of transplantation (non-significant habitat effect). Others such as brood mortality showed a highly significant habitat effect and a significant interaction, indicating that there was differences in the environmental effect between populations. Life span and brood number were the only traits (excluding maximum size) that had a significant habitat effect without any interaction, suggesting that the effect of environment was equal across all populations. Overall, amphipods raised in wood debris substrate had a shorter life span and produced fewer broods than those raised in bank and *Fucus* (which were statistically indistinguishable), indicating that the life span and brood number of bank and *Fucus* animals were reduced when they were raised in wood debris substrate. Population - habitat interactions were evident for size at reproductive maturity, brood mortality, age at reproductive maturity, egg size, fecundity, weight of newly released juveniles and mortality to reproductive size. Multiple range tests on these data (Table 3) helped elucidate the nature of the genotype - environment interaction. In all cases except mortality to reproductive maturity there was one population, raised in all three substrates, that was grouped separately from the other two, indicating a rigidly fixed trait in that population. That

Table 3. Results (F values) of anova on reciprocal transplant. For sources Population, Habitat and Generation (numerator df=2) p=.05 at F=3.0; two factor interactions (df=4) p=.05 at F=2.37; three factor interaction (df=8), p=.05 at F=1.94. Example of notation used in multiple range test: WD/F wood debris amphipods raised in Fucus substrate; situations within the same brackets are not significantly different ($p > .05$); transplant situations are listed from smallest value for that trait (top) to the largest (bottom). Trait abbreviations: BRD = brood number; DEV = development time; RESZ = size at reproductive maturity; LFSP = life span; BRDMRT = brood mortality; MXSZF = maximum size of females; AGERP = age at reproductive maturity; EGSZ = egg size; INTCLU = interclutch interval; EGGS = fecundity; MXSZM = maximum size of males; JUVWT = weight of newly released juveniles; JUVMRT = mortality prior to reproductive size.

Source of Variation	BRD	DEV	RESZ	LFSP	BRDMRT	MXSZF	AGERP	EGSZ	INTCLU	EGGS	MXSZM	JUVWT	JUVMRT
Population	215.5	477.9	256.3	2014.0	3460.3	6.7	11890.8	65.7	907.7	843.2	58.5	94.3	119.3
Habitat	3.1	2.1	61.1	6.3	2998.1	10.6	44.7	3.9	2.3	141.8	92.7	6.2	12.6
Population x Habitat	1.4	<1	3.1	2.1	831.8	1.6	43.2	2.4	<1	8.8	14.4	3.7	62.8
Generation	<1	<1	1.9	<1	<1	<1	2.7	<1	1.2	<1	1.5	2.2	2.0

Multiple Range Tests on Population - Habitat Interaction

RESZ	BRDMRT	AGERP	EGSZ	EGGS	JUVWT	JUVMRT
(WD/WD)	(WD/F WD/BK)	(WD/F)	(F/WD)	(WD/WD)	(F/WD)	(WD/WD)
(WD/BK BK/WD WD/F)	(WD/BK WD/WD)	(WD/WD BK/F)	(F/BK)	(WD/BK)	(F/BK)	(BK/BK WD/F)
(BK/BK)	(F/F)	(WD/BK BK/BK)	(BK/BK BK/WD BK/F F/F)	(WD/F)	(BK/WD)	(WD/F F/F WD/BK/F)
(F/WD F/BK)	(BK/F)	(BK/WD)	(WD/F WD/BK)	(F/WD)	(BK/BK BK/F F/F)	(WD/BK BK/F BK/WD)
(F/F)	(BK/BK)	(F/BK)	(WD/WD)	(BK/WD F/BK)	(WD/F WD/BK)	(F/BK F/WD)
	(F/BK)	(F/F)		(F/BK BK/BK)	(WD/WD)	
	(BK/WD)	(F/WD)		(F/F BK/F)		
	(F/WD)					

trait in the other two populations was subject to considerable phenotypic modulation. For example, brood mortality of the wood debris population was lower, in all substrate types, than both *Fucus* and bank populations (multiple range test grouped the wood debris population separately from the other two populations irrespective of the substrate in which the animals were raised); animals from *Fucus* on the other hand, had relatively low brood mortality in their native substrate but very high brood mortality in wood debris; and amphipods from bank had relatively high brood mortality in their native substrate which increased even more when they were transplanted into wood debris. Another similar example is egg size, where amphipods from the wood debris population produced larger eggs than those of amphipods from the other two populations, irrespective of the substrate in which they were raised. Most traits that were phenotypically modulated still displayed some genetic basis for the observed variation. For example, fecundity of *Fucus* and bank amphipods in wood debris substrate, although significantly lower than in their native substrates, was not as low as wood debris animals in any of the substrates.

These data and this analysis suggest a genetic basis for the observed variation, in all populations, for brood number, development time, life span interclutch interval; and for the following combinations of traits and populations: large size at reproductive maturity in amphipods from *Fucus*, smaller size in those from wood debris and bank; large egg size in wood debris, smaller size in bank and *Fucus*; low brood mortality in animals from wood debris, high brood mortality in those from bank; later age of reproductive maturity in *Fucus*, much earlier in bank and wood debris; low fecundity in wood debris, higher fecundity in bank and *Fucus*. Size of newly released juveniles is very closely correlated with egg size (compare

multiple range test results for EGSZ and JUVWT) and therefore, there is reason to believe that larger juveniles are a consequence of producing larger eggs.

Results of the transplants for the additional six populations were very similar to those involving the Squamish populations (Table 4) suggesting there is also a genetic basis to the trait variation in these additional populations. In other words, the population effect (synonomous with genotype) was greater than the habitat effect (synonomous with environment). Since the transplants in this case were only maintained for a single generation, their is no generation factor in the analysis of variance. I am assuming the non - significant generation effect typical of the transplants involving the Squamish populations is also applicable to these populations ie. maternal effects due to differences in nutritional status of the mothers from each of the respective habitats is not important in explaining the variation. This seems a reasonable assumption when one considers the similarity of the anovas involving these additional populations with those involving the Squamish populations. Several traits had habitat effects without any interaction; amphipods raised in wood debris substrate had lower overall RESZ in both analyses, as was the case for LFSP and fecundity in the lower mainland analysis. The interclutch interval of animals from the Queen Charlottes was significantly longer in *Fucus* substrate. The interaction data sets were similar to the original Squamish populations.

Interpopulation Crosses

The results of the interpopulation crosses verified that there was a genetic basis to the observed variation (Table 5 & 6); the mean value of F_1 traits generally lied somewhere between that of the parents (occasionally above one parent), with standard deviations similar or only slightly larger than that observed in the parents.

Table 4. Anova results (F values) on reciprocal transplant of Queen Charlottes and Lower Mainland (southwestern-corner of B.C.) populations. For sources population and habitat p=.05 at F=3.05 and for the interaction p=.05 at F=2.42. Trait abbreviations and conventions are the same as in Table 3.

A) Estuary in Queen Charlottes

Source of Variation	BRD	DEV	RESZ	LFSP	BRDMRT	AGERP	EGSZ	INTCLU	EGGS	JUVWT	JUVMRT
Population	134.8	59.8	27.2	793.8	927.6	4361.7	27.0	229.9	311.5	49.8	26.2
Habitat	1.3	<1	12.2	<1	846.2	9.9	<1	3.5	41.8	8.1	3.3
Population x Habitat	1.7	<1	<1	2.1	228.2	5.7	<1	2.2	2.9	4.4	13.9

Multiple Range Tests on Population - Habitat Interaction

BRDMRT	AGERP	EGGS	JUVWT	JUVMRT
(WD/F WD/BK WD/WD)	(WD/F WD/WD)	(WD/WD)	(F/WD BK/WD)	(WD/WD)
(WD/WD F/F)	(WD/WD BK/F WD/BK)	(WD/BK WD/F)	(BK/WD F/BK)	(BK/BK WD/F F/F WD/BK BK/F)
(F/F BK/F)	(WD/BK BK/BK)	(F/WD)	(F/F BK/F BK/BK)	(WD/BK BK/F BK/WD)
(BK/BK)	(BK/BK BK/WD)	(F/BK BK/WD BK/BK)	(WD/F)	(BK/WD F/BK)
(F/BK)	(F/BK)	(F/F BK/F)	(WD/BK)	(F/BK F/WD)
(BK/WD)			(WD/WD)	
(F/WD)				

Table 4 cont'd.

B) Lower Mainland and Vancouver Island

Source of Variation	BRD	DEV	RESZ	LFSP	BRDMRT	AGERP	EGSZ	INTCLU	EGGS	JUVWT	JUVMRT
Population	89.9	123.0	16.6	780.9	1311.7	3799.6	35.6	181.0	290.7	58.7	23.9
Habitat	<1	<1	11.2	3.2	713.7	10.4	<1	<1	37.0	4.3	1.4
Population x Habitat	<1	<1	1.6	<1	205.7	11.3	<1	<1	2.0	2.9	9.3

Multiple Range Tests on Population - Habitat Interaction

BRDMRT	AGERP	JUVWT	JUVMRT
(WD/F WD/BK WD/WD)	(WD/F WD/WD)	(F/WD)	(WD/WD)
(F/F)	(WD/WD WD/BK)	(F/BK)	(BK/BK WD/F WD/BK F/F BK/F)
(BK/F)	(BK/F BK/WD BK/BK)	(BK/WD F/F)	(WD/BK F/F BK/F BK/WD)
(BK/BK)	(F/BK)	(BK/BK BK/F F/F)	(BK/WD F/BK)
(F/BK)	(F/F)	(WD/F WD/BK)	(F/BK F/WD)
(BK/WD)	(F/WD)	(WD/WD)	
(F/WD)			

Table 5. Measurements of life history traits in F_1 hybrids formed by crossing wood debris populations with BKSQ; males of each cross are listed first. Location acronyms: BKSQ, bank population from Squamish; WDSQ, wood debris population from Squamish; WDCR, wood debris population from Campbell River; WDQC, wood debris population from the Queen Charlottes.

Trait	BKSQ/BKSQ	BKSQ/WDSQ	WDSQ/BKSQ	WDSQ/WDSQ	WDCR/WDCR	BKSQ/WDCR	WDCR/BKSQ	WDQC/WDQC	BKSQ/WDQC	WDQC/BKSQ
BRD	3.4(1.2)	3.7(1.2)	3.7(1.2)	3.8(1.2)	3.9(1.3)	3.8(1.3)	3.8(1.2)	4.1(1.3)	4.4(1.4)	4.3(1.4)
DEV	17.74(1.90)	20.37(2.32)	20.16(2.24)	21.25(2.48)	21.32(2.55)	20.55(2.44)	20.50(2.60)	19.51(2.22)	20.19(3.22)	19.97(3.06)
LFSP	265.9(27.6)	297.7(29.9)	298.9(29.2)	307.7(29.6)	297.8(26.8)	293.9(27.2)	295.1(28.0)	310.3(28.8)	315.8(34.1)	311.5(32.4)
EGSZ	.458(.081)	.511(.072)	.508(.066)	.524(.061)	.533(.059)	.527(.068)	.522(.060)	.564(.062)	.556(.077)	.557(.070)
EGGS	61.3(7.0)	51.7(6.5)	53.5(7.4)	43.6(5.7)	42.5(5.3)	48.4(6.4)	49.6(6.2)	40.7(5.1)	40.4(5.6)	41.9(5.9)
INTCLU	13.9(3.3)	16.5(4.1)	16.2(4.3)	17.7(3.7)	17.2(3.5)	15.8(3.9)	16.0(4.3)	17.9(4.1)	15.7(4.3)	15.2(3.9)
ABSFIT	54.0(18.7)	24.2(17.8)	31.5(19.3)	40.1(22.2)						

Table 6. Measurements of life history traits in F_1 hybrids resulting from interpopulation crosses involving FSQ; (*Fucus* population from Squamish); A. BKSQ X FSQ; B. WDSQ X FSQ. Males of each cross are listed first.

A.

Trait	BKSQ/BKSQ	FSQ/BKSQ	BKSQ/FSQ	FSQ/FSQ
BRD	3.4(1.2)	4.9(1.5)	5.2(1.7)	5.4(1.3)
DEV	17.74(1.90)	15.55(1.82)	15.29(1.74)	15.26(1.64)
LFSP	265.9(27.6)	409.4(35.9)	413.6(37.6)	422.7(37.4)
AGERP	176.7(11.3)	295.7(12.9)	299.7(13.4)	311.9(12.1)
EGSZ	.458(.081)	.471(.071)	.474(.077)	.486(.067)
EGGS	61.3(7.0)	54.6(8.3)	49.3(7.9)	58.8(5.3)
INTCLU	13.9(3.3)	7.8(2.8)	7.4(3.0)	6.4(2.6)

B.

Trait	WDSQ/WDSQ	WDSQ/FSQ	FSQ/WDSQ	FSQ/FSQ
BRD	3.9(1.3)	5.5(1.8)	5.7(1.7)	5.4(1.2)
DEV	20.12(2.53)	18.77(2.27)	18.89(2.44)	15.20(1.54)
LFSP	301.0(25.7)	432.1(39.8)	440.3(38.2)	418.9(34.1)
AGERP	167.6(9.41)	305.3(18.0)	308.3(20.1)	315.7(12.8)
EGSZ	.526(.062)	.519(.065)	.522(.070)	.491(.060)
EGGS	44.7(6.7)	49.2(7.0)	43.4(7.2)	64.6(5.7)
INTCLU	18.1(3.9)	4.7(3.1)	5.0(2.6)	5.2(2.2)

Substantial departure from intermediacy was evident for most life history traits in F_1 hybrids (Table 7). This was not due to maternal effects, since reciprocal crosses had approximately the same values. Explanation for the deviation from the mid - parent value thus lies in dominance. The direction of dominance in crosses involving the wood debris populations from Campbell River and the Queen Charlottes was in the same direction as for the cross between the Squamish bank and wood debris populations but the degree of dominance was different (Table 5 & 7). The Queen Charlotte wood debris population exhibited overdominance or near complete dominance for brood number, life span and egg size. Egg size and fecundity varied reciprocally in the F_1 of crosses between the wood debris populations and the Squamish bank population. Fecundity was generally below the mid - parent value, with directional dominance towards larger eggs. Greater directional dominance in egg size was associated with an increased deviation in fecundity in the opposite direction (towards 0).

Crosses between the Squamish bank and *Fucus* populations exhibited dominance for brood number, life span, delayed age at reproductive maturity and faster development time. The egg size of hybrids was approximately intermediate, indicating additive genetic effects for eggs of this size. All crosses exhibited dominance in brood number and life span; overdominance, or complete dominance, was evident for these traits in crosses between wood debris and *Fucus*. Longer life span was also associated with delayed age at reproductive maturity in hybrids involving *Fucus*; age at reproductive maturity and life span covaried to the extent that the largest deviations from the mid - parent value in age at reproductive maturity were associated with the largest values in life span.

Table 7. Deviation from mid-parent values of life history traits in crosses between wood debris populations and BKSQ, and between the three Squamish populations.

	BRD	DEV	LFSP	AGERP	EGSZ	EGGS	INTCLU
BKSQ/WDSQ	.75	.75	.76		.80	.46	.68
WDSQ/BKSQ	.75	.69	.79		.76	.56	.60
BKSQ/WDCR	.80	.78	.88		.92	.31	.58
WDCR/BKSQ	.80	.77	.91		.86	.38	.64
BKSQ/WDQC	1.43	1.38	1.12		1.05	- .01	.45
WDQC/BKSQ	1.29	1.26	1.03		.97	.06	.32
BKSQ/FSQ	.90	.01	.94	.91	-.57	-3.80	.13
FSQ/BKSQ	.75	.12	.91	.88	.43	-1.68	.19
WDSQ/FSQ	1.07	.73	1.11	.93	.80	.23	- .04
FSQ/WDSQ	1.20	.75	1.18	.95	.89	- .06	- .01

Selection Test

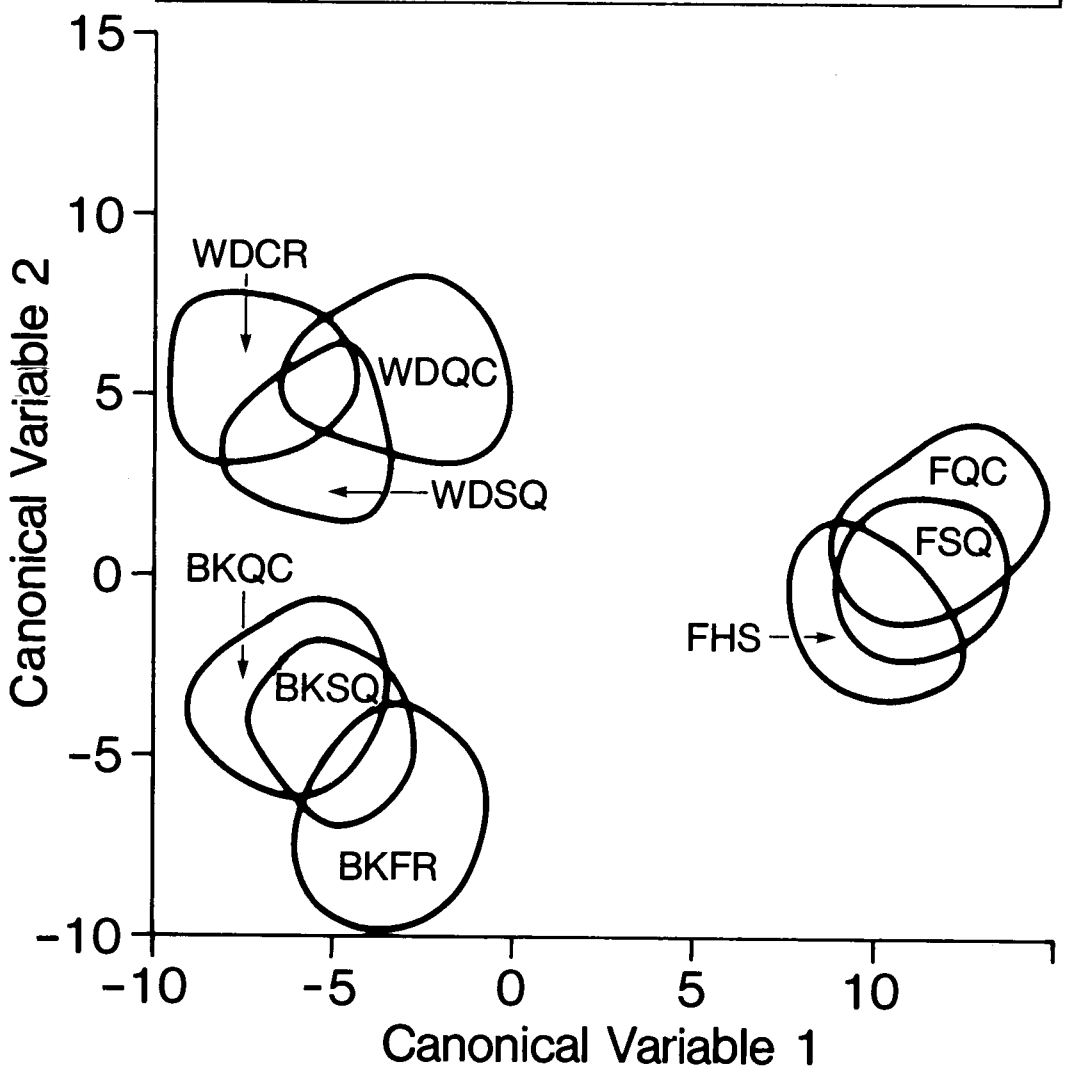
Multiple discriminant analysis (including all traits present in Table 3, except maximum size of males and females) separated the nine populations into three distinct life history phenotypes, grouping all the populations from a particular habitat type together (Fig. 6; Bartletts X^2=407.4 $p<<0.0001$). Generalized distances were greater for inter - habitat comparisons than for intra - habitat comparisons (Fig. 6). A classification matrix indicated that animals not classified into the population from which they arose were always classified into a group of similar habitat type. These data suggest that there was selection of an overall life history phenotype in each of the habitat types concerned.

Life history variables were entered into the discriminant analysis in the following order: brood mortality, age at reproductive maturity, egg size, life span, interclutch interval, size at reproductive maturity, brood number, development time, fecundity, weight of newly released juveniles and mortality to reproductive size, indicating their relative importance in group discrimination. The traits most correlated with canonical discriminant function I and II were the same as those determined in the analysis of the original Squamish populations.

Nested anova results indicated there was a significant habitat effect for all traits; brood number, F=87.1; development time, F=178.1; size at reproductive maturity, F=96.3; life span, F=757.9; brood mortality, F=750.7; age at reproductive maturity, F=4915.6; egg size, F=50.6; fecundity, F=674.8; interclutch interval, F=348.5; weight of newly released juveniles, F=138.8; mortality to reproductive size, F=19.1. All traits except egg size, weight of newly released juveniles and mortality to reproductive size, were grouped separately in multiple range tests. *Fucus* and bank habitats were grouped together in these exceptions. Crosstabulation of the

Fig. 6. Canonical plot and matrix of Mahalanobis generalized distances from multiple discriminant analysis of all nine populations; $p < .0001$ for all generalized distances; BKSQ, BKFR and BKQC refer to bank populations from Squamish, Fraser River and Queen Charlottes respectively; WDSQ, WDCR and WDQC, wood debris populations from Squamish, Campbell River and the Queen Charlottes respectively; FSQ, FHS and FQC, *Fucus* populations from Squamish, Howe Sound and Queen Charlottes respectively.

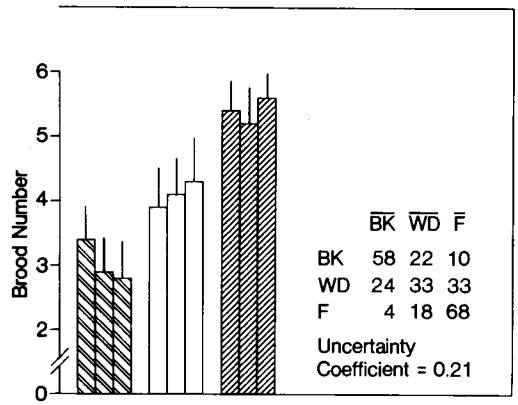
	BKSQ	BKFR	BKQC	WDSQ	WDCR	WDQC	FSQ	FHS
BKSQ								
BKFR	15							
BKQC	5	24						
WDSQ	114	117	88					
WDCR	118	149	83	9				
WDQC	159	173	95	17	14			
FSQ	439	336	339	476	415	312		
FHS	363	281	326	321	352	241	6	
FQC	324	281	318	387	385	290	4	8

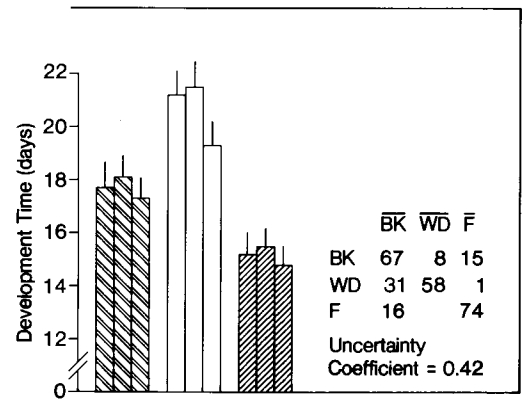


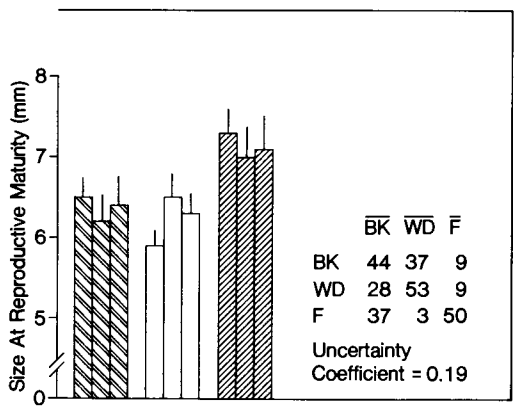
cluster data for each trait indicated amphipods were generally classified into the habitat type from which they arose (Fig. 7; chi - square for each crosstabulation data set was significant at $p<0.0001$). This was particularly evident for traits such as life span, age at reproductive maturity and fecundity, resulting in relatively high uncertainty coefficients for those traits. These coefficients reflect the ability to predict the value of a particular trait in all three habitats. In instances where the uncertainty coefficient was relatively low it does not necessarily however, indicate a total absence of predictive ability; in most such cases there was at least one habitat type in which the classification was clear (ie. amphipods originating from a particular habitat type were classified in that habitat), the absence of any pattern in the other two resulted in the low coefficient.

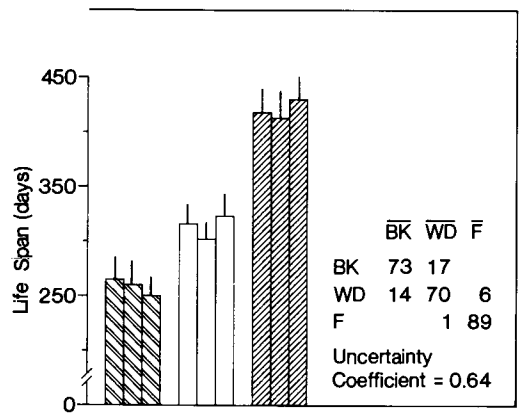
These crosstabulation data and the results of the nested anova suggest selection of all the traits, in at least one of the habitat types. For example, the only obvious pattern for egg size was that animals from wood debris produced larger eggs. Analysis of other traits however, suggest selection in all three habitat types (eg. life span). These data must be viewed in concert with the results of the transplantation experiment. For example, brood mortality of females from bank and wood debris raised in *Fucus* substrate was lower than in their respective native substrates, while brood mortality of females from *Fucus* increased when transplanted to other substrates, indicating that there must be something about *Fucus* substrate that induces low brood mortality and therefore, the low brood mortality typical of *Fucus* amphipods cannot reliably be considered due to natural selection. A conservative list of the traits that the evidence (crosstabulation data and nested anova, concomitant with considering the degree to which a trait is modulated by transplantation) suggests selection of, would be the following:

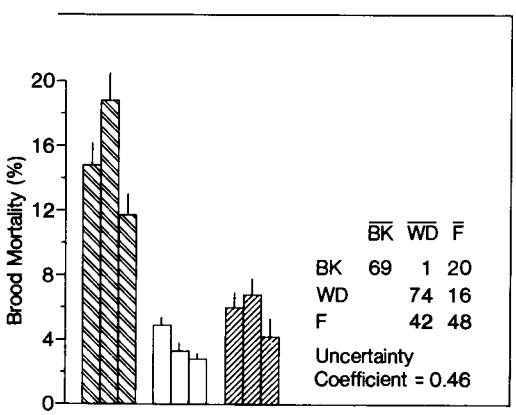
Fig. 7. Means (with 2 x SE) of life history traits from populations included in the selection test, with crosstabulation data and uncertainty coefficients.

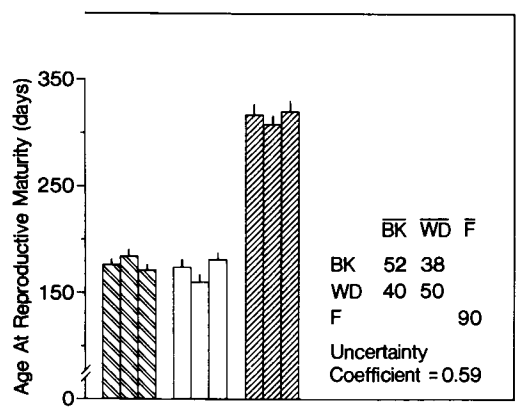

Brood Number
0
2
3
4
5
6
BK WD F
BK 58 22 10
WD 24 33 33
F 4 18 68
Uncertainty
Coefficient = 0.21

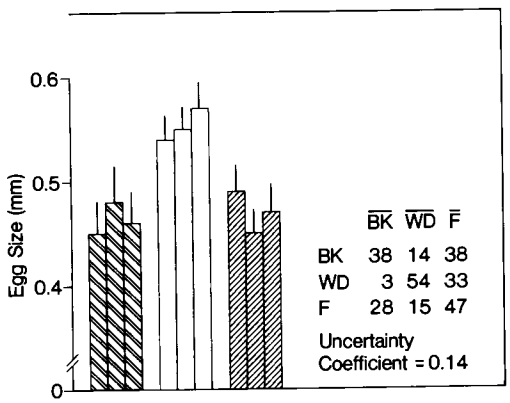

Development Time (days)
0
12
14
16
18
20
22
BK WD F
BK 67 8 15
WD 31 58 1
F 16 74
Uncertainty
Coefficient = 0.42

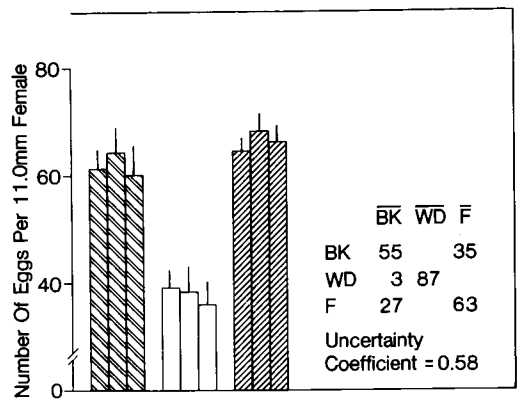

Size At Reproductive Maturity (mm)
0
5
6
7
8
BK WD F
BK 44 37 9
WD 28 53 9
F 37 3 50
Uncertainty
Coefficient = 0.19

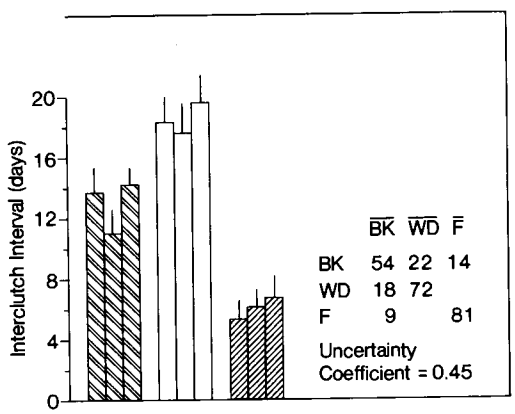

Life Span (days)
0
250
350
450
BK WD F
BK 73 17
WD 14 70 6
F 1 89
Uncertainty
Coefficient = 0.64

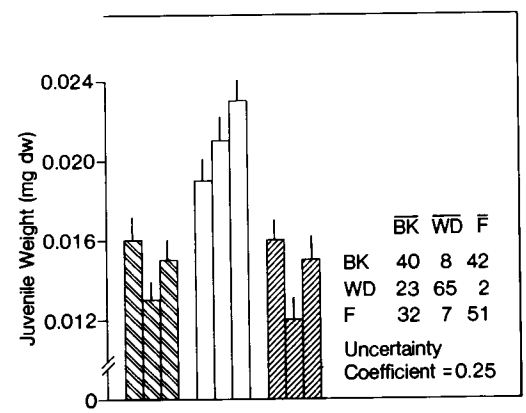

Brood Mortality (%)
0
4
8
12
16
20
BK WD F
BK 69 1 20
WD 74 16
F 42 48
Uncertainty
Coefficient = 0.46

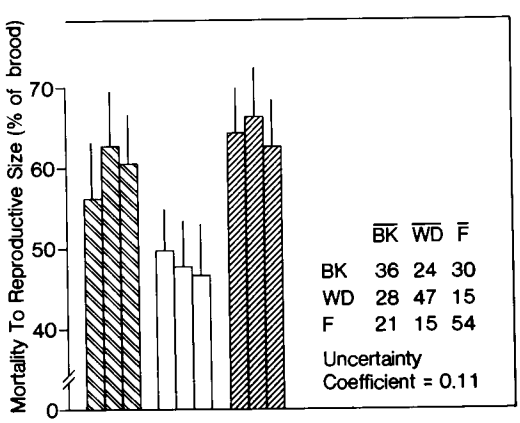

Age At Reproductive Maturity (days)
0
150
250
350
BK WD F
BK 52 38
WD 40 50
F 90
Uncertainty
Coefficient = 0.59

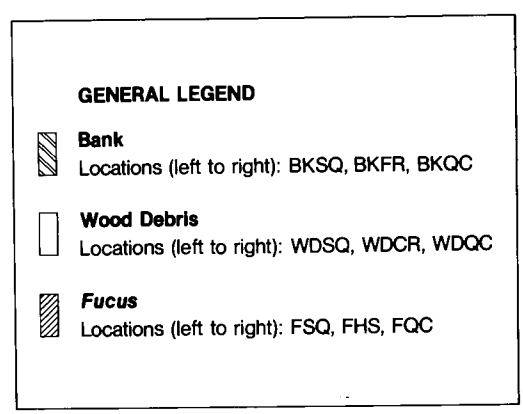

Egg Size (mm)
0.6
0.5
0.4
0
BK WD F
BK 38 14 38
WD 3 54 33
F 28 15 47
Uncertainty
Coefficient = 0.14


Number Of Eggs Per 11.0mm Female
80
60
40
0
BK WD F
BK 55 35
WD 3 87
F 27 63
Uncertainty
Coefficient = 0.58


Interclutch Interval (days)
20
16
12
8
4
0
BK WD F
BK 54 22 14
WD 18 72
F 9 81
Uncertainty
Coefficient = 0.45


Juvenile Weight (mg dw)
0.024
0.020
0.016
0.012
0
BK WD F
BK 40 8 42
WD 23 65 2
F 32 7 51
Uncertainty
Coefficient = 0.25


Mortality To Reproductive Size (% of brood)
70
60
50
40
0
BK WD F
BK 36 24 30
WD 28 47 15
F 21 15 54
Uncertainty
Coefficient = 0.11


GENERAL LEGEND
Bank
Locations (left to right): BKSQ, BKFR, BKQC
Wood Debris
Locations (left to right): WDSQ, WDCR, WDQC
Fucus
Locations (left to right): FSQ, FHS, FQC

1. low, medium and high brood number in bank, wood debris and *Fucus* respectively.

2. slow, medium and fast development time in wood debris, bank and *Fucus* respectively.

3. large size at reproductive maturity in *Fucus*.

4. short, medium and long life span in bank, wood debris and *Fucus* respectively.

5. low brood mortality in wood debris.

6. delayed age at reproductive maturity in *Fucus*.

7. large egg size in wood debris.

8. low fecundity in wood debris; high fecundity in bank and *Fucus*.

9. short interclutch interval in *Fucus*; long interval in wood debris.

A caveat is necessary at this point: selection would not act specifically (ie. selection *for*, discussed by Sober 1984) to reduce fecundity or brood number and presumably not to increase development time and interclutch interval. Such fitness components should contribute positively to total fitness and therefore be under directional selection to increase. This list merely summarizes the results to this point, indicating which traits are genetically fixed (or nearly so) and closely correlated with habitat type (therefore suggesting selection *of*, Sober 1984).

A summary of the array of life history traits typical of each habitat appears in Table 8.

Table 8. Summary of Eogammarus confervicolus life history traits typical of each habitat.

Habitat	Summary of Life History Characteristics
Wood Debris	Slow development time and long interclutch interval, concomitant with an increased number of broods and longer life span (relative to bank), result in an extended reproductive period. Population is bivoltine. Fewer, larger eggs with low brood mortality, resulting in fewer, larger offspring with high survivorship.
Bank	Short life span and consequent low brood number, an intermediate interclutch interval and development time, result in a short reproductive period. Population is bivoltine. Many, smaller eggs with high brood mortality result in a relatively large number of smaller offspring with low survivorship.
Fucus	Delayed reproduction and longer life span with consequent larger size at reproductive maturity. Increased number of broods with rapid development and short interclutch interval. Population is univoltine. Many, smaller eggs with low brood mortality result in many, smaller offspring with very low survivorship.

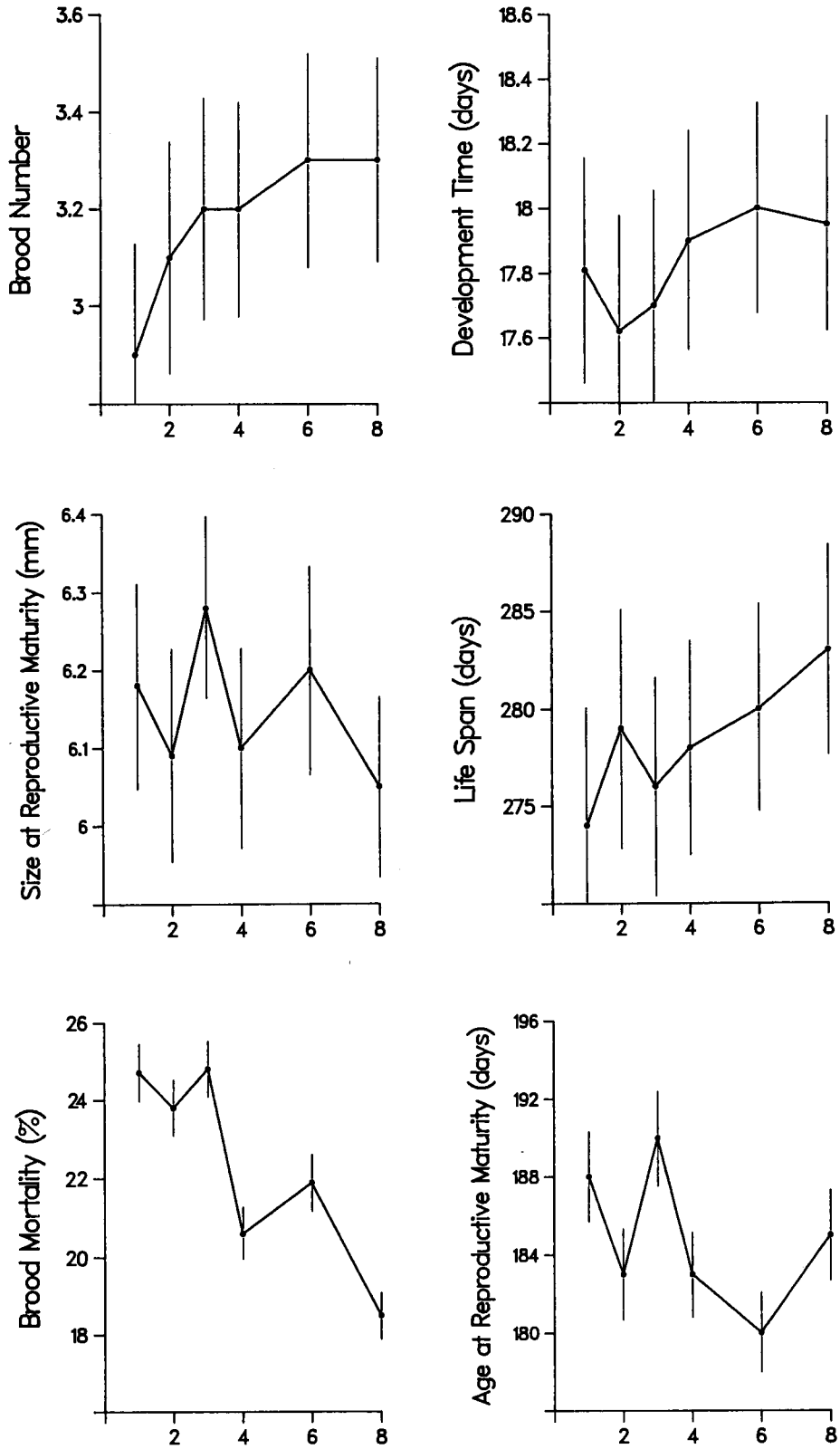
Selection Experiment

A significant response to selection, towards a wood debris life history phenotype was evident in most of the measured characters (Figure 8 and Table 9). Exceptions were: RESZ, AGERP, INTCLU and DEV (although the response to selection in INTCLU and DEV was not quite significant, the trend was towards values typical of the wood debris life history phenotype). Selection of fecundity and egg size were similar in the replicate and principal tanks (replicate: EGGS,-1.0096; EGSZ,.0039), while no selection was evident in the control (significance of regression for EGGS: .8895; EGSZ: .7763), indicating the response to selection was not a laboratory artifact. The significant response to selection in absolute fitness suggested the amphipods were adapting to the new substrate through changes in the life history traits.

Mating Success

Between 75 and 97% of the matings were successful (Table 10). Lower percentage mating success (75-80%) was evident in intrapopulation crosses as well as between populations (see WDSQ x WDSQ and WDCR x WDCR for example). Results of the anovas indicated there was no effect of location (F=1.53), habitat (F=2.36), generation (F=2.13), or source of mother or father (F=.3) on mating success. These data and this analysis suggest that amphipods from all nine populations, comprise a single species.

Fig. 8. Response of life history traits to selection (raising bank amphipods in wood debris substrate); mean and SE are shown for each trait over the 8 or 9 generation selection period. The slope and significance of regression lines through these means are presented in Table 9. For purposes of comparison I provide the following pairs of numbers, referring to the means of bank amphipods in bank substrate and wood debris animals in wood debris substrate for each life history trait; the first number of each pair refers to bank amphipods, the second to wood debris amphipods. Trait abreviations correspond with a left to right reading of the figure. BRD: 3.4, 3.9; DEV: 17.7, 21.2; RESZ: 6.5, 5.9; LFSP: 265, 316; BRDMRT: 14.8, 4.9; AGERP: 176, 174; EGSZ: .46, .54; EGGS: 61.3, 39.2; INTCLU: 13.7, 18.3; JUVWT: .016, .019; JUVMRT: 56.2, 49.7.


Brood Number
3.6
3.4
3.2
3
Development Time (days)
18.6
18.4
18.2
18
17.8
17.6
Size at Reproductive Maturity (mm)
6.4
6.3
6.2
6.1
6
Life Span (days)
290
285
280
275
Brood Mortality (%)
26
24
22
20
18
Age at Reproductive Maturity (days)
196
192
188
184
180
2
4
6
8
Generation

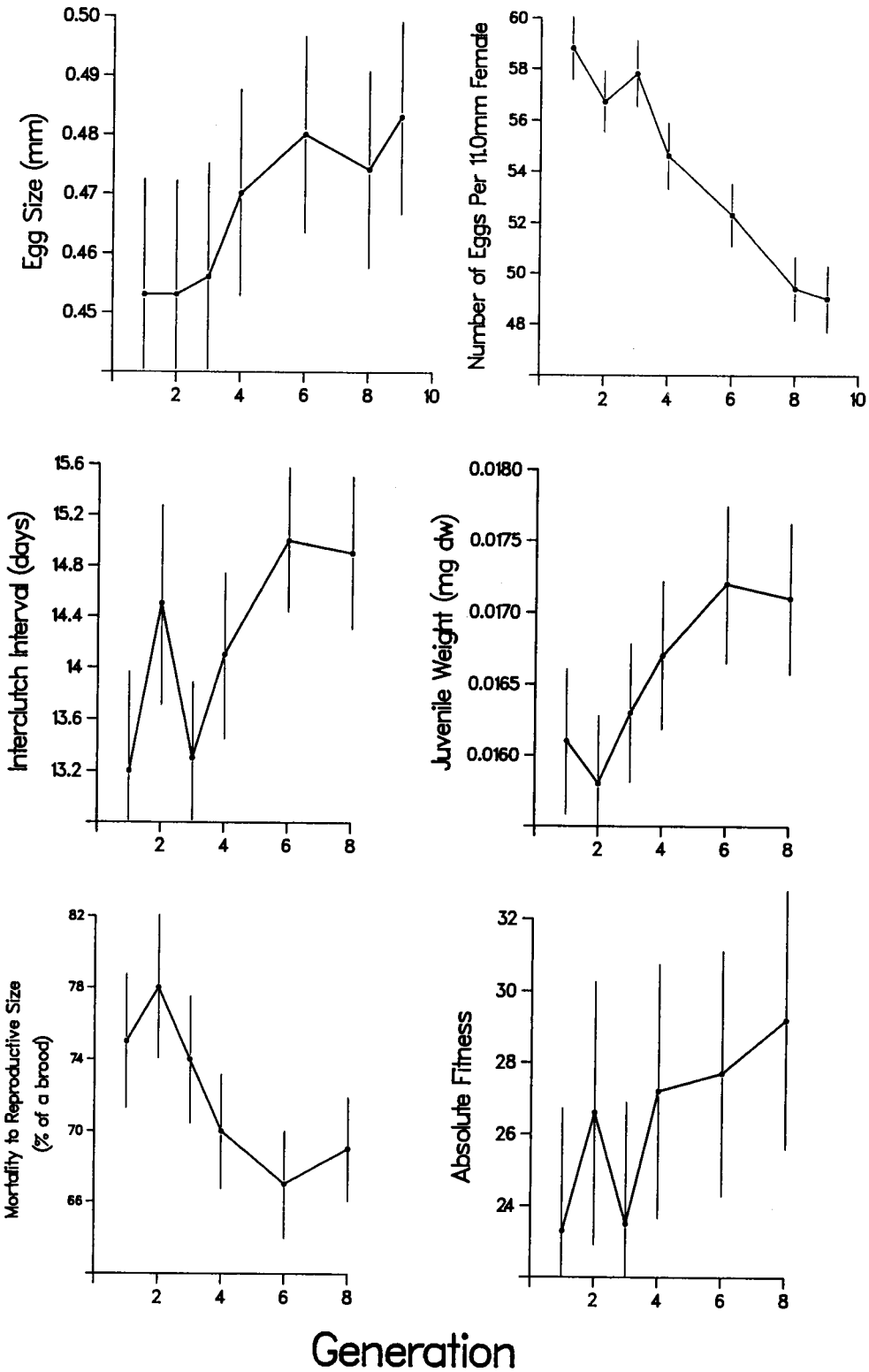

Egg Size (mm)
0.50
0.49
0.48
0.47
0.46
0.45
2
4
6
8
10
Number of Eggs Per 11.0mm Female
60
58
56
54
52
50
48
2
4
6
8
10
Interclutch Interval (days)
15.6
15.2
14.8
14.4
14
13.6
13.2
2
4
6
8
Juvenile Weight (mg dw)
0.0180
0.0175
0.0170
0.0165
0.0160
2
4
6
8
Mortality to Reproductive Size
(% of a brood)
82
78
74
70
66
2
4
6
8
Absolute Fitness
32
30
28
26
24
2
4
6
8
Generation

Table 9. Results of selection experiment.

Response to selection is the slope of a regression line fitted to the means of each trait at generations 1-4,6 and 8 (and for EGSZ and EGGS, at generation 9 as well); standard errors are in brackets. Trait abbreviations the same as in Tables 1 and 3 with the addition of CLUVOL = clutch volume; ABSFIT = absolute fitness.

Trait	Significance of Regression	Response to Selection
BRD	.0257	.0500 (.0144)
DEV	.0921	.0421 (.0224)
RESZ	.4839	
LFSP	.0211	1.0588 (.2872)
BRDMRT	.0228	-.8441 (.2346)
AGERP	.4076	
EGSZ	.0039	.0039 (.0008)
INTCLU	.0806	.2265 (.0974)
EGGS	.0001	-1.2778 (.1143)
JUVWT	.0138	.0002 (.00005)
JUVMRT	.0417	-1.3235 (.4478)
CLUVOL	.0091	-.4165 (.0881)
ABSFIT	.0420	.7529 (.2547)

Table 10. Mating success (percentage of crosses producing offspring) of reciprocal crosses involving the nine populations; top row of each set is success of parental cross, bottom row is F_1 x F_1. Parents from populations listed across the top of the matrix are female. Codes for populations are the same as in Figure 6.

	BKSQ	BKFR	BKQC	WDSQ	WDCR	WDQC	FSQ	FHS	FQC
BKSQ	86.6	85.5	94.1	94.3	84.9	81.7	84.8	85.8	84.5
	95.9	88.7	81.8	88.1	88.9	83.2	86.3	86.5	90.3
BKFR	96.8	87.5	92.7	79.2	94.1	94.6	97.8	95.7	82.1
	97.1	81.6	86.0	79.3	93.7	85.1	95.2	85.3	94.0
BKQC	94.0	89.0	92.9	80.9	88.2	82.9	86.4	77.4	96.3
	82.6	85.6	95.4	95.8	87.5	82.6	76.9	80.6	84.9
WDSQ	80.1	93.4	93.3	89.8	92.0	76.5	84.1	90.7	96.7
	83.2	78.6	81.1	78.7	89.2	85.9	82.2	80.5	80.2
WDCR	81.2	96.5	97.2	95.6	78.0	86.3	88.2	86.9	96.8
	91.2	97.2	84.1	97.9	81.3	85.4	95.3	96.4	85.4
WDQC	80.8	94.6	96.8	85.8	90.3	84.5	91.3	84.2	76.6
	86.9	82.0	88.3	79.1	95.1	88.3	79.8	78.1	80.8
FSQ	79.5	83.1	89.2	77.1	76.9	90.6	78.0	82.9	84.5
	95.5	76.9	78.7	93.9	91.7	80.7	86.8	79.0	87.0
FHS	89.7	77.1	91.7	84.0	95.1	92.9	83.2	86.0	93.9
	93.4	80.8	81.8	82.1	87.3	75.8	95.8	86.1	89.0
FQC	86.6	79.5	80.6	80.4	76.2	92.8	79.6	82.7	97.1
	86.0	90.9	84.8	90.6	81.5	82.6	82.4	84.2	93.3

DISCUSSION

Ecotypes refer to adaptation within a species to different environments. Since the absolute fitness of amphipods in this study is greatest in their native substrate they are adapted to existence in that substrate. The results of the inter - population crosses suggest they can satisfactorily be considered to comprise a single species. Genetically based variation in life history traits, concomitant with population specific DNA polymorphisms, indicates the populations are genetically distinct. If one chooses however, to refer to them as life history ecotypes, then the life history traits are the adaptations that suit the environment. Since there was selection towards a wood debris life history phenotype after eight generations of transplant, concomitant with an increase in absolute fitness, the strong inference is that the intraspecific variation in life history traits represent habitat specific adaptations.

The term ecotype has generally been applied to plants; an example is heavy metal tolerance of certain plant species in response to mining activities (Antonovics and Bradshaw 1970), in which local adaptations occur on an extremely fine scale. Adaptations are solutions to ecological problems. In the case of this mining example, the problem and the solution are clear so it is appropriate to term these plants heavy metal tolerant ecotypes. My selection experiment manipulated one variable: substrate, which must then be a selective agent for the wood debris life history phenotype. One way in which wood debris substrate differs from *Fucus* and bank is that it supports a much reduced biomass of the microbial epiphytes used as food by *E. confervicolus*. McKeag (1983) presented evidence indicating that wood chips are a relatively poor substrate for bacterial growth. She determined bacterial densities, supported from various organic substrates, under varying inorganic nutrient regimes.

The substrates included two algal species, *Ulva lactuca* and *Fucus vesiculosus*, the vascular plant *Zostera marina*, and wood chips. Wood chips supported bacterial densities one to three orders of magnitude less than the algae and one to two orders of magnitude less than *Zostera marina*. The addition of inorganic nutrients greatly increased bacterial densities supported by the seaweeds but had no effect on bacterial biomass supported by wood chips. Preliminary results of my own, obtained by sonnicating wood chips and *Fucus* fragments and observing the resulting suspended material using epifluorescent microscopy, support McKeag's findings. Estuarine wood chips supported much lower bacterial, diatomaceous, and fungal biomasses than did *Fucus* blades (Appendix II). Most of the food energy in wood debris is tied up as lignified cellulose. Life history theory predicts that a resource scarce habitat should select for fewer, larger offspring (Stearns 1976); this is what I find in wood debris habitats and this is one of the responses to the selection experiment. I submit that the nutrient depletion typical of wood debris substrate is one of the ecological problems encountered by amphipods in wood debris habitats, to which the life history solution is fewer, larger offspring. As such, it may be appropriate to term these life history phenotypes, life history ecotypes.

Several other hypotheses do exist however, to explain the partitioning of reproductive effort into offspring of different sizes. These include the relative importance of predator swamping, competitive demands on young, stability of environmental conditions, r/k selection and habitat availability (Stearns 1976). Wood debris substrate *per se* is apparently sufficient selective agent to explain the partitioning of reproductive effort (and several other life history traits) in wood debris amphipods. Since I did not perform a selection experiment in *Fucus* and bank the importance of these respective substrates as selective agents is less certain. The

discriminant function analysis (selection test) suggests that *Fucus* and bank habitats are selective agents, but any one, or a combination, of the above mentioned selective mechanisms could be operating.

The evidence suggests a possible cause - effect relationship between the nutrient depletion typical of wood debris substrate (selective agent) and larger juveniles (target of selection). I have no reason to believe a similar cause - effect relationship exists for the remaining traits. I suggest therefore, they are correlated responses. Several of these traits are undoubtedly phenotypically correlated (or simply a consequence of another trait or combination of traits): eg. EGSZ and JUVWT; BRDMRT with EGSZ and EGGS; JUVMRT with a combination of traits, particularly JUVWT. Others may be genetically correlated.

Major components of fitness should contribute positively to fitness and thus be under directional selection to increase. Brood number should therefore, be under directional selection to increase (within certain constraints) in all habitats. The selection experiment indicated it was possible to select for increased brood number in bank amphipods, simply by raising them in wood debris substrate and the selection test indicated the wood debris life history phenotype produced, on the average, an additional brood. Perhaps there is a physiological constraint on increase in brood number in bank habitats, set by the high reproductive effort per brood, which is alleviated in wood debris substrate, due to the drop in fecundity. An inverse correlation between a fitness component and fitness is indicative of a negative correlation between that component and some other fitness character (Lande and Arnold 1983). An indication of such an inverse correlation was evident in the selection experiment, between fecundity and absolute fitness (Figure 8). This finding is in direct contrast to results from the Giesel laboratory (Giesel 1979; Giesel and

Zettler 1980; Giesel et al. 1982), which suggest that fitness components are positively correlated. In fact Giesel and Zettler (1980, p. 302) go so far as to say: "all components of fitness are positively correlated: within limits, a strain which is "fit" in one respect is superior in other aspects as well". My results are in agreement with those of Hiraizumi (1961), Simmons et al. (1980) and Rose and Charlesworth (1981b), in which the enhancement of one fitness component depresses another; as EGSZ increased, EGGS dropped. This negative correlation (measurements of EGGS and EGSZ came from the same individuals) could be genetic or phenotypic. If fecundity was solely a physical consequence of egg size, due to the restrictions imposed on females through the size of their brood pouch or energy available for egg production, then one would expect clutch volume to be consistent with increasing egg size. The selection experiment (Table 6) however, indicated there was a reduction in clutch volume with increasing egg size. This does not exclude the possibility of a phenotypic correlation between these two life history characters but is evidence suggesting it may not be the sole explanation. The chief cause of genetic correlations is pleiotropy (Falconer 1981). Negative genetic correlations have been termed antagonistic pleiotropy and suggested as a genetic mechanism behind trade-offs between life history fitness components (Simmons et al. 1980; Charlesworth 1980; Rose and Charlesworth 1981a,b; Rose 1984; Rose et al. 1987). Trade-offs are an integral part of the reproductive effort life history theory of Williams (1966) and Gadgil and Bossert (1970) and the prevalent assumption is that they are a common phenomenon in the evolution of life histories. Such trade-offs can be analagous to Stearns' tactics. For example, fewer, larger progeny is a trade-off between fecundity and egg size and may represent a life history tactic, the purpose of which is to produce larger offspring, that have an increased chance of survival in a nutrient poor

environment. The results of the interpopulation crosses between wood debris and BKSQ indicated a complete reciprocity between EGSZ and EGGS; the larger the egg size the fewer eggs were produced. This could be a phenotypic correlation or may represent pleiotropic gene action which is antagonsitic between dominant alleles for egg size and fecundity.

Another combination of traits which could be physically tied are development time and egg size. Many studies of marine invertebrates have demonstrated longer development times in animals with larger eggs (McLaren 1966; Corkett 1972; Steele 1977; Hart and McLaren 1978; Woodward and White 1981; Clarke 1982; Lonsdale and Levinton 1985). A common explanation for this correlation is that gas exchange across larger eggs is slower and this necessitates a lower metabolic rate (Corkett 1972; Clarke 1982). McLaren (1966) however, reported marked differences in development times of copepods producing similar sized eggs. My results from bank and *Fucus* are similar to McLaren's findings. Egg size cannot be the sole determinant of development time: *E. confervicolus* from the *Fucus* habitat have the same sized eggs as those from bank but have much faster development time. Rapid development time appears to be selected independently of egg size in these animals. The interpopulation crosses suggested faster development time typical of the *Fucus* amphipods was dominant, however, when crossed with wood debris amphipods the larger eggs typical of the F_1 was associated with an increase in development time. It seems likely that longer development time is a physical consequence of producing larger eggs, however, I cannot rule out the possibility of negative pleiotropic effects between large eggs and developmental rate. Fast development time, on the other hand, appeared to be inherited independently.

Quantitative genetics theory predicts that directional selection should result in directional dominance, with the direction of dominance toward higher fitness. There should be no directional dominance in traits selected for intermediate optima (Fisher 1958; Kearsey and Kojima 1967; Jinks 1979; Mather and Jinks 1982). The results of my selection experiment indicated directional selection of bank amphipods when raised in the laboratory simulated wood debris habitat and the interpopulation crosses revealed directional dominance for many of the life history characters typical of wood debris amphipods. These two pieces of evidence suggest the wood debris life history phenotype is the consequence of directional selection.

The evidence suggests selection of life history traits in all three habitats. In fact, the evidence suggests selection of a suite of life history characters in each habitat. This could be due to a combination of phenotypic and genetic correlations between traits. An alternative to the selection argument is that the three populations of each habitat type arose from the same ancestor, possessing characteristics allowing it to survive in the respective substrate. If the life history traits were selectively neutral they would not have stayed the same after amphipods colonized the respective locations (The degree of independence between these populations and these selection events is the subject of Chapter 2). Another factor to consider is that these environments represent three, of a large number of possible habitats for this animal. If amphipods from another habitat (eg. cobble - boulder beaches) were grouped with bank animals in the discriminant function analysis, then one would conclude that the bank habitat was not a unique selective agent for those life history traits. Unfortunately, I do not have detailed data from any additional habitats. I cannot rule this out as a possibility, however, it still remains a fact that a female's absolute fitness is greatest in her native substrate, indicating adaptation to that

substrate. Such reservations are obviously not necessary for the wood debris situation since the selection experiment indicates wood debris substrate is a selective agent for several of the life history traits. Furthermore, the selection experiment indicates that a wood debris phenotype can be selected from a bank phenotype and all available evidence indicates that prior to log storage in Squamish, this area was a bank habitat. Remnant patches of *Carex* (including the embankment that forms the perimeter of such a marsh) are present in this log storage area and *Carex* rhizomes are present all across the mudflat (top of the old marsh), at anywhere from 5 cm to 1.0 m below the surface. It was not then, a case of a log debris habitat being created over an otherwise barren mudflat and amphipods subsequently colonizing, but instead the perturbation of an already existing bank population, resulting in change in the population trait distributions (method VI outlined by Endler 1986 for detecting selection).

Logging activities first started in the vicinity of the Squamish estuary about 1900. An aerial photograph clearly shows active log storage in the estuary in 1932. The area prior to log storage was a bank habitat, as already explained. I surmise then, the Squamish wood debris life history phenotype diverged from a bank phenotype over a period of about 75 - 100 years (150 - 200 generations). The response to selection will depend to a large extent on the additive genetic variance present within the base population. Because I do not know the proportion selected for any of the traits in the selection experiment, I cannot determine the selection differential and therefore cannot calculate realized heritabilities. I can however, assume considerable additive genetic variance for these fitness components in the base population. For example, if I make the unreasonably rigid assumption of truncation selection of brood number with the top 20% selected, the resulting heritability (calculated as

$R=1/2h^2S$; 1/2, because measurements pertain only to females; Falconer 1981) is .26; top 40%, the heritability is .40. Similar calculations for life span yield heritabilities of .24 at 40% and .48 at 70%. Fitness can be thought of as an index by which natural selection simultaneously selects for the major components. Fisher's fundamental theorem of natural selection states that the increase of fitness in one generation equals the additive genetic variance of fitness; therefore, the selection response in absolute fitness (Table 6) expressed as a proportion of the total phenotypic variance of absolute fitness (at generation 8) yields the heritability (0.33). Fisher's theorem is thought to apply to populations not in equilibrium; those that are in equilibrium should have no heritable variation in fitness (Falconer 1981). The bank population is presumably at or near equilibrium (existed as long as the delta; very limited immigration and emmigration; trait distributions did not change significantly during the period of this study). Transplanting amphipods into wood debris substrate may have altered the relative weighting of fitness components, resulting in some additive genetic variance for fitness, allowing the population to respond to natural selection. A possible example in this regard, concerns the fact that larger juveniles have much higher survivorship in wood debris substrate, and since size is not of similar importance in bank substrate, some additive genetic variance for offspring size might be expected in the equilibrium population. This variance component would not be a significant contributor to additive variance in fitness within bank substrate, but would be in wood debris. Istock (1983) offers the speculation that populations possess "potential" variation in polygenic traits, that may only be released by environmental change, through altered effects on gene expression. Such variation could provide the central genetic basis for adaptation. It is possible that substantial additive variance for all these fitness components, so

evident upon transplantation, may also be present in the bank equilibrium. Many empirical studies have demonstrated considerable heritable variation in fitness components (see Istock 1983 for review). Lande (1975) argued that high levels of additive variance could be maintained by mutation - selection balance even in the presence of strong stabilizing selection. Rice (1988) has presented a model which suggests that substantial amounts of additive genetic variance for fitness should be present within natural populations due to mutation - selection balance. Rose (1982) presented a model which indicates that antagonistic pleiotropy between fitness components could in principle act to maintain additive genetic variance. The results of my selection experiment indicate there is considerable additive variance in life history traits and the antagonsitic selection response between EGGS and EGSZ concomitant with the reciprocity observed between these two characterisitcs in interpopulation crosses suggests the possibility of antagonistic pleiotropy between components (I should emphasize however, that I have not ruled out the possibility these two life history characters are simply phenotypically correlated).

At the estimated increase in absolute fitness per generation, the current absolute fitness level of the wood debris phenotype would be reached in 40 - 50 generations. Since I know the Squamish wood debris habitat has existed for at least 100 generations, the inference is that fitness has remained somewhere near the current level for about 50 generations. This implies that the mean fitness is no longer increasing because the genetic composition of the population, following a period of directional selection, has reached a new equilibrium. Interestingly, the absolute fitness of wood debris and bank amphipods in their respective native substrates is very similar (Table 2), suggesting that mean fitness in these bivoltine populations evolves to a certain maximum or optimum and remains there; possibly set by

carrying capacity. *Fucus* animals on the other hand have an absolute fitness approximately double that of bank and wood debris. Delaying reproduction in *Fucus* means that animals are larger at reproductive maturity and thus have a greater reproductive value. Increased size in *Fucus* results in a greater increase in fecundity, relative to bank and wood debris (slope of fecundity vs size regression is greater than bank or wood debris). This increase in fecundity, concomitant with the additional broods, yields the greater absolute fitness.

Doyle and Hunte (1981a,b) subjected an estuarine amphipod to selection for high population growth rates by providing a laboratory environment that was uncrowded, constant, with excess food. In 26 generations the intrinsic rate of population growth was 72% greater than a control population (wild) due to heritable changes in fecundity, survivorship and age at maturation. They hypothesized the mechanism for this rapid adaptation to an altered environment, was high additive genetic variance for fitness components in the source population. A similar process would seem to be operating in the present situation.

The major difference in life history phenotype between *Fucus* and the other two types was age at reproductive maturity and length of life span. A bivoltine cycle has been selected in bank and wood debris and a univoltine cycle in *Fucus*. Despite the fact *Fucus* amphipods were approximately the same size as those from bank and wood debris they did not reproduce in the winter and delayed reproduction until the following spring. Theories on the evolution of life span all suggest that it can be altered by natural selection and represent an adaptive feature of an animal's life history (Haldane 1941; Williams 1957; Hamilton 1966; Emlen 1970; Rose and Charlesworth 1980). Williams (1957) was the first to suggest pleiotropic genetic control of senescence. Most artificial selection experiments have focused on

modifying life span by selecting for early or late reproduction. Generally, an increase in longevity has accompanied selection for late reproduction. *Fucus* animals in this study possessed very delayed reproduction and an associated increase in life span (age at reproductive maturity was about 150 days later with approximately 60% greater life span than bank amphipods and about 30% greater than wood debris). Luckinbill et al. (1984) approximately doubled the life span of *Drosophila melanogaster* (in 13 generations) by selecting for late reproduction. My results indicate that selection for delayed reproduction and longer life span in *Fucus* was beyond the range present in either bank or wood debris populations (see crosstabulation data in Figure 7). Longer life span was associated with delayed age at reproductive maturity in hybrids involving *Fucus*; AGERP and LFSP covaried to the extent that the largest values for deviation from mid - parent in AGERP were associated with the largest values in LFSP. My results are in agreement with the generally held belief that longevity is genetically determined (see for eg. Rose and Charlesworth 1980, 1981b; Luckinbill et al. 1984) and are thus contrary to the studies of Lints et al. (1979), Lints and Hoste (1974) and the discussions by Lints (1978, 1983), in which they conclude longevity is determined by maternal effects. My data suggest dominant genetic effects are responsible for longer life span. My selection experiment and selection test suggested life span is independent of age at reproductive maturity in wood debris substrate. Perhaps there is a threshold point beyond which AGERP and LFSP interact. Hiraizumi (1961) described a situation in *Drosophila melanogaster* where rate of development was negatively correlated with fertility when development rate was faster than a particular level and positively when it was slower than this level, suggesting a threshold is of some importance in determining the interrelationships between these life history characters.

Speculations on causal factors for delayed reproduction would include: much increased adult survivorship relative to bank and wood debris (Stanhope and Levings 1985) and an increased reproductive value with increasing age relative to bank and wood debris. Whatever the cause, there are considerable consequences on population dynamics and this in turn has implications on gene flow between the groups. The univoltine cycle of the *Fucus* population means that the winter adults of bank and wood debris cannot form hybrids with *Fucus*. Adult amphipods from the bank and wood debris winter generation would not form mating pairs in laboratory crossing experiments with *Fucus* animals collected at the same time. Hybrids can be formed however, in summer months. Wood debris and *Fucus* populations were separated by only a few hundred metres of mud flat in the Squamish and Queen Charlotte sites. Since the life history phenotypes represent a suite of adaptations to each of the environment types, the potential disruption of adaptive traits by forming hybrids would presumably result in reduced fitness. I do not know how much juvenile or adult amphipods move about at high tide but if there were significant exchange between *Fucus* and wood debris habitats, the annual size frequency distributions from Squamish would not have been as regular as that observed (Stanhope and Levings 1985, reproduced in Appendix III). The fact I can identify population specific DNA RFLPs (see Figures 4 and 5) indicates that there must be little gene flow between any of the Squamish populations.

Perhaps the most surprising result of this study, was the complete reversal in relative fitness of wood debris and bank amphipods when they were raised with *Fucus* animals; their absolute fitness was significantly less when raised in *Fucus* substrate than in their native habitats, however, due to the differences in voltinism over the same period of time, bank and wood debris animals had greater overall

reproductive success than *Fucus* amphipods. Since selection acts on relative fitness and not absolute fitness, I was left with the paradoxical question of why bank or especially wood debris amphipods (because of their proximity) had not replaced *Fucus* animals in *Fucus* substrate. Some form of competition prevented this from happening when the two other life history phenotypes were raised with *Fucus* animals; a greater competitive ability of *Fucus* animals in their native substrate would appear to have resulted in the relative fitness figures. Competitive ability has been used as a measure of relative fitness in strains of *Drosophila* (Ayala 1970; Yamazaki 1984). In these studies however, the various strains have roughly comparable reproductive potentials. In the present case, the superior reproductive potential of bank and wood debris animals is surpassed by some competitive ability possessed by *Fucus* amphipods. Whatever the nature of this ability it is likely an important factor in preventing wood debris amphipods from taking over the nearby *Fucus* environment.

This amphipod species has gone through significant evolutionary change without speciation. Why should intraspecific differentiation be so evident in this species? Recently, extensive differentiation in allozymes have been demonstrated between amphipod populations (eg. Bulnheim and Scholl 1981; Bulnheim and Scholl 1982; Bulnheim 1985; Siegismund 1985; Siegismund et al. 1985; McDonald 1987). Most crustaceans produce swimming larvae or release eggs. Planktonic dispersal should reduce inter - population genetic variation by increasing gene flow and there is evidence to support this (eg. Berger 1973; Winans 1980; Johnson and Black 1984). Gooch (1975) and Crisp (1978) have concluded there is generally an inverse correlation between dispersal capability, as measured by length of pelagic larval life, and the extent of divergence between conspecific populations.

Development in amphipods on the other hand, is direct and therefore they have very limited dispersal capabilities. Estuarine amphipods have an additional dispersal problem: suitable habitat occurs as scattered pockets (estuaries or brackish water bays) separated by large expanses of high salinity water. Bulnheim and Scholl (1981) found electrophoretic enzyme variation to be much greater in an estuarine gammarid than in a closely related marine species, suggesting diminished gene flow, due to the brackish water requirements of the one species, lead to genetic separation of local populations. Bulnheim (1985) has found significant differences in allozymes between populations of the euryhaline amphipod *Gammarus tigrinus* along the coasts of Germany and the Netherlands; interestingly, this amphipod species has colonized this area only within the last 25 - 30 years, indicating that the observed differences have developed over a relatively rapid period. The stream dwelling amphipod *Gammarus minus* provides somewhat of an analog to *E. confervicolus*. Holsinger and Culver (1970) described three forms of G. *minus* throughout the Mid - Appalachians, which they regard as ecophenotypes. Each phenotype is found in a different habitat: extensive cave systems, smaller more isolated caves and springs. Gooch and Hetrick (1979) found highly differentiated allele frequencies among the populations comprising these ecophenotypes, which they suggested was due to the separation of these distinct habitats by barriers to migration. Ecophenotypes were not more genetically similar than populations at large, suggesting the ecotypic variation did not reflect separate colonization events from three distinct ancestors. Intertidal and estuarine isopods brood their young in a brood pouch and genera such as *Jaera* and *Sphaeroma* have population specific colour polymorphisms (see Hedgecock et al. 1982 for review). In contrast to the peracarid crustaceans, decapods have highly vagile juveniles and adults, produce dispersing larvae and possess very little

geographic variation among conspecific populations (eg. Tracey et al. 1975; Lester 1979).

Evidence also exists for locally differentiated sub - populations. Borowsky et al. (1985) presented evidence that *Gammarus palustris* was locally differentiated into sub - populations within a large brackish water bay and that this subdivision was related to feeding preferences of different genotypes. At a number of localities along the European Atlantic coast, *Jaera marina* exists in several morphological forms, each occupying a narrow intertidal band (Bocquet 1954). These groups will interbreed in the laboratory but apparently do not hybridize in nature. Boquet suggested these forms are not just microgeographic ecotypes, but incipient or full species. Goedmakers (1980) presents life history evidence suggesting microgeographic races of three gammarid amphipod species along the same river (some stations less than 1 km apart).

My results, along with a growing body of evidence (see especially Doyle and Hunte 1981a,b), indicate that adaptation in marine invertebrates can occur rapidly enough to be of interest not only to evolutionary biologists, but also to marine ecologists concerned with physical alteration of nearshore and estuarine habitats. As Doyle and Hunte (1981a) suggested, genetic local races of marine invertebrates may actually be the norm rather than the exception. I submit, that rapid changes in life history trait distributions may be quite common in estuarine peracarid Crustacea due to strong selection, high heritabilities for fitness components (perhaps supported through antagonistic pleiotropy, mutation - selection balance or present as "potential" variation), and little mixing of gene pools.

CHAPTER II

GENOTYPE ANALYSIS

INTRODUCTION

In the previous chapter I presented correlational evidence for selection of life history traits in bank and *Fucus* habitats and much stronger evidence for selection in wood debris. A germane question is whether animals from similar habitat types arose from members of the same genetic race or are the observed differences the consequence of selection on independent genotypes? The results of my selection experiment, concomitant with the fact the wood debris habitat in Squamish was once a *Carex* marsh, suggest that the wood debris phenotype diverged from a bank phenotype and thus the genotype of WDSQ might be expected to be more similar to that typical of Squamish bank populations than to a wood debris population in Campbell River, for example.

Recombinant DNA techniques are ideally suited to an examination of differences between population genotypes, due to the great precision to which one can fingerprint the groups. Starch gel electrophoresis of proteins is often hampered because of the absence of polymorphic loci. Restriction endonuclease analysis of mitochondrial (mt) DNA has proven a valuable population tool, partly due to its accelerated rate of nucleotide substitutions relative to nuclear DNA (Brown et al. 1979). Unfortunately the animal biomass required to isolate sufficient mtDNA for analysis of these amphipod populations made this approach unfeasible. Restriction analysis of large nuclear genomes is ordinarily not possible because the number of resulting fragments is too large to permit resolution. This problem can be overcome if restriction digests are combined with hybridization using radioactively labelled cloned fragments that allow examination of a few homologous fragments at a time. Restriction fragment length polymorphisms (RFLPs) associated with these cloned fragments can be used as a measure of genetic relatedness (see Rose et al. 1982 for

an example of the use of this methodology in genetic mapping studies, and Natvig et al. 1987 as a precident for its use in phylogenetics). Using this approach I have examined the genetic relatedness between the nine populations included in the earlier selection test and use this information to decide whether the observed variation in life history traits represents selection of independent genotypes.

MATERIALS AND METHODS

DNA Preparation

DNA was prepared after the method outlined by Bender et al. (1983). One gram of amphipods (25 - 50 individuals; either alive or frozen in liquid nitrogen) were added to 20 ml of 0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris HCl, 0.05 M EDTA (pH 9.1) with 0.5% SDS and 1% diethyl pyrocarbonate. The amphipods were ground quickly in a mortar containing laboratory sand and the slurry was incubated at 65°C for 30 mins. Then 3 ml of 8 M potassium acetate was added, the mixture was kept at 0°C for 30 min, followed by centrifugation for 5 min at 10000 g. The supernatant was recovered and mixed with an equal volume of ethanol and allowed to precipitate at -20°C. DNA was removed with a glass rod, washed twice with 70% EtOH and resuspended in 10 mM Tris, 1 mM EDTA pH 7.5 (1 X TE).

DNA was purified by banding in cesium chloride/ethidium bromide (28 grams of CsCl, 1 ml EtBr in final volume of 40 ml 1 X TE). Centrifugation was for 24 hr at 45000 rpm and 25°C in a Beckman Vti 50 rotor. The single visible band was removed through the side of the tube with a slanted wide bore hypodermic syringe. Ethidium bromide was removed with NaCl and H_2O saturated isopropanol. The CsCl concentration was lowered by adding 2 volumes of H_2O. DNA was precipitated with EtOH at -20°C overnight, washed twice with 70% EtOH and resuspended in 1 X TE (pH 7.5). Individual extractions were thoroughly mixed to obtain working samples (after verifying that DNA from each extraction would cut properly with restriction endonucleases). This was done to assure that each sample was as representative of the population genotype as possible. Resulting concentrations were between 0.25 and 0.65 ug/ul. Samples were stored with chloroform at 4°C.

Gel Electrophoresis, Southern Transfers and Hybridization, Cloning and Probe Preparation

DNA from each of the nine populations was digested with the restriction endonucleases EcoR I, Hind III and occasionally Bgl II, Pst I and BamH I. Complete digestion was generally accomplished by incubating 20 - 30 units of enzyme / 5ug of DNA for 3 - 4 hours at 37°C. The conditions required for complete digestion of each sample were determined from visual inspection of restriction digests, photographed using a 260nm transilluminator and by co-restricting equal amounts of amphipod and lambda DNA. The duration and enzyme concentration necessary to resolve the lambda into its respective fragments was evaluated as the appropriate restriction conditions for that sample. Digests were run overnight in 0.7% agarose gels, except when resolution of similar sized fragments was necessary, when the concentration of agarose was increased. The size of restriction fragments were estimated by comparison with EcoR I - Hind III digests of lambda DNA.

Following electrophoresis, DNA was transfered to nitrocellulose filters (Schleicher and Schuel) by the method of Smith and Summers (1980). Transfers were left overnight. Filters were baked in a vacuum oven for 1.5 - 2 hr at 80°C. Blots were pre-hybridized in 4 X SET (1 X SET is 0.15 M NaCl, 30 mM Tris (pH 8.0), 2 mM EDTA), 0.1% sodium pyrophosphate, 0.1% SDS, 25 mM sodium phosphate (pH 6.8) and 5 X Denhardt's solution (1 X Denhardt's solution is 0.02% BSA, 0.02% Ficoll, 0.02% polyvinylpyrolidine) (Kovesdi and Smith 1985) for 2-5 hrs at 65°C. Hybridization was carried out in a fresh aliquot of the above solution, along with a heat denatured, radiolabelled probe (see below), overnight at 65°C. Hybridized blots were washed 3 - 5 times in 0.2 X SET, 0.2% SDS at 65°C, dried

and then autoradiographed for 1 - 10 days with Kodak BB1 or XAR-5 and Lightning Plus intensifying screens at -70°C.

Genomic DNA from each of the three Squamish populations and the plasmid PUC19 were digested to completion with EcoR I, Hind III, BamH I and Pst I, ligated and transformed into *E. coli* JM83. The ratio of insert to vector was 5:1. Transformed cells were plated on nutrient agar plates containing XGAL, IPTG and ampicilin. Plasmid DNA preparations were according to Davis et al. (1980). Clones obtained in this manner were screened to obtain probes of unique sequence. The screening process involved the detection of inserts that would not hybridize with any of the other cloned fragments (hybridization conditions: 5 X SSPE, 0.3% SDS at 65°C; washed in 0.2 X SSPE, 0.3% SDS at 65°C; 1 X SSPE = 0.18 M NaCl, 0.010 M sodium phosphate and 1 mM EDTA pH 7.4). Over 100 unique probes were obtained in this fashion, of which 41 (chosen randomly) were used in this analysis, ranging from 1.0 to 5.9 kb, with an average of 2.7 kb. Probes were prepared by purifying the inserts on low melting point agarose (Langridge et al. 1980) and labelling with ^{32}P by nick translation (Rigby et al. 1977) to specific activities of 5 - 30 X 10^7 cpm/ug.

All the probes used in this analysis are repeat sequences. This I concluded from hybridizing nick translated genomic DNA to the cloned inserts; those inserts that hybridize must be repeated throughout the genome of the nick translated sample.

Data Collection Scheme

Restriction digests of DNA from each population, bound to nitrocellulose were hybridized against each of the cloned fragments. Polymorphisms were determined by comparison of autoradiographic banding patterns between the nine

populations. Results were summarized as molecular weights of hybridized bands. A pairwise cataloging of restriction fragments shared among populations provided measures of genetic distance. Pairwise distance matrices were constructed from values obtained using the following relationships:

$$(1)\ \text{Distance Index} = 1 - \frac{\#\ \text{fragments common to both populations}}{\#\ \text{of fragments in both populations}}$$

$$(2)\ p = 1 - \frac{[-F + (F^2 + 8F)^{1/2}]^{1/n}}{2}$$

$$\text{standard deviation} = \frac{[p(1-p)]^{1/2}}{N}$$

where p = number of base substitutions per nucleotide

F = fraction of fragments shared between 2 populations

n = number of base pairs recognized per enzyme cleavage site

N = the total number of base pairs in sites cleaved by the restriction enzymes used (total number of fragments in both populations X 6 (hexameric enzymes used only; nucleotides in common cleavage sites are counted only once).

The latter equation was derived by Upholt (1977) (for an example of application of this method see Avise et al. 1979). Both distance estimates yield the same relative picture (since equation 2 is based on 1); I include the latter estimate because data of this nature is commonly expressed in terms of percentage of nucleotides substituted and because it provides a measure of standard error. Upholt's derivation of this formula is based on the assumption that fragment changes arise by base substitution. I cannot reliably assume this to always be the case in the following data set (deletion and insertion events may be responsible for some RFLPs),

therefore, the accuracy of estimates for percentage nucleotide substitution should be viewed with some caution. It does however, provide an additional means of expressing the data, that may be preferable to some. I hasten to mention that any use of this data to estimate evolutionary rates would be inappropriate since the probes are randomly cloned repeat sequences and not the single copy DNA upon which such estimates are based (see Britten 1986 and Nei 1987).

The distance values were subjected to the unweighted pair group clustering procedure (UPGMA; Sneath and Sokal 1973) and the resulting dendrograms provided a pictorial representation of the relationships between groups.

Probe Nomenclature

Each probe is designated by a combination of letters referring to the species it was cloned from, the population genotype from which the clone was obtained, the enzyme used in cloning and a number. For example: ECFE1 refers to probe number one, cloned from *Eogammarus confervicolus* (EC), *Fucus* population (F, BK, WD refer to Squamish *Fucus*, bank and wood debris populations respectively), in the EcoR I site (E, H, B, and P refer to EcoR I, Hind III, BamH I and Pst I respectively) of Puc19.

Eogammarus oclari

Eogammarus oclari is the only other species in this genus (Bousfield 1979). It is much less ubiquitous than *E. confervicolus* but the two species can occasionally be found sympatrically. In order to provide a relative picture of the genotypic variation within the species compared to that between species I examined the genomic DNA of *E. oclari* using a few of the probes with conserved restriction sites

within *E. confervicolus*. DNA methods were the same as for those involving *E. confervicolus*. Individuals were examined under a dissecting microscope for the spinal arrangements on the telson and third uropod used as diagnostic structures for differentiating *E. oclari* from *E. confervicolus* (see Bousfield 1979). *Eogammarus oclari* specimens were frozen in liquid nitrogen until sufficient biomass had accumulated for DNA extraction.

RESULTS AND DISCUSSION

The molecular weights of hybridized bands across all nine populations for all 41 probes are catalogued in Table 11. Examples of hybridized blots appear in Figure 9. Most of the probes are scattered repeat sequences (eg. probes ECBKB3, ECBKP4), some are tandem repeats (different enzymes yield a fragment of the same molecular weight; eg. ECFE2, ECBKH25), others hybridized to tandem sequences and to scattered sequences in the same digest (eg. ECBKB5, ECBKE12, ECWDE18). Scattered repeats were more variable than tandems, including those of the same sequence. Most probes only hybridized to 1-3 fragments, which may be due to their relatively small size (larger probes such as ECBKP4, ECBKB5, ECWDE9 hybridized to more fragments). Several probes revealed fragments common to all bank and wood debris populations which differed from those of *Fucus* (eg. ECBKH6, ECBKP11).

The distance matrix, based on pairwise cataloging of restriction fragments appears in Table 12; the resulting dendrogram appears in Figure 10 (dendrograms using both types of data yielded the same picture). The standard errors for distance estimates involving nucleotide substitutions were all extremely small: 2 X SE = .0002 - .0004. Since I am interested in the relative genetic distance between populations, I am concerned with estimates of "mean" population genotypes. Each DNA extraction involved 25 - 50 animals and after purification individual extractions were thorougly mixed to obtain the working samples. I assume therefore, my samples represent the "mean" population genotype. Obtaining this data through an analysis of individuals would not have been feasible, since the amount of purified DNA obtained from an individual amphipod was barely enough for one gel. On the occasions I looked for intrapopulation polymorphisms using DNA from single

Table 11. Genomic fragments with homology to cloned inserts (sizes given in kilobase pairs). See methods for an explanation of probe nomenclature. Enzyme used in each genomic digest is indicated.

Probe Name and Insert Size	Enzyme	FSQ	FHS	FQC	BKSQ	BKFR	BKQC	WDSQ	WDCR	WDQC
ECFE1, 1.0	EcoR I	9.5 1.0	9.5 1.0	9.5 1.0	9.5 1.0	9.5 1.0	9.5 1.0	9.5 1.0	9.5 1.0	9.5 1.0
	Hind III	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
	Bgl II	9.5 5.0 4.5	9.5 5.0 4.5	9.5 5.0 4.5	9.5	9.5	9.5	9.5	9.5	9.5
ECFE2, 2.7	EcoR I	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
	Hind III	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
	Bgl II	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
ECBKB3, 4.6	EcoR I	6.8 3.1	6.8 3.1	5.2 3.6	10.2 2.8	14 4.1	9.4 5.9	10.2 3.0	15 12	4.8 3.4
	Hind III	7.5 4.9	7.5 4.9 4.0	7.5 5.5 1.4 1.0	17 11 1.9	9.1 8.3	7.0 4.1 1.4 1.2	14 11 4.6	6.4 5.1 1.6	5.2 4.1
ECBKP4, 5.9	EcoR I	18 9 4.4 4.0 3.2	14 9 5.6 4.0 3.2 2.1	16 9 7.9 4.0 2.8 2.0	20 12 7.2 6.1 5.9 2.0 1.1 0.7	5.9 5.1 4.3 2.2 1.3	7.7 7.1 5.3 1.2 1.0 0.8	11 8.9 7.2 5.9 2.0 1.1	6.6 6.1 1.5 1.3 0.9	9.3 7.1 1.0 0.8
	Hind III	11 10 4.1	17 12 11 10 5.8 4.7	20 13 7.8 3.4 3.1 1.4	9.8 6.0 4.9 4.7 4.1 2.9 2.0 1.9 1.35 1.30	7.0 4.0 3.7 3.3 2.8 1.6 0.8	7.8 6.6 2.5 1.7 0.9	9.0 6.0 5.0 4.7 4.1 2.9 2.1 2.0	6.8 2.4 1.25 1.1 1.05 1.0	10.5 9 1.65 1.55 0.95

Probe Name and Insert Size	Enzyme	FSQ	FHS	FQC	BKSQ	BKFR	BKQC	WDSQ	WDCR	WDQC
ECBKB5, 5.4	EcoR I	20	20	17	10	8.8	8.8	8.8	8.8	11
		14	14	8.8	8.8	5.9	8.2	7.7	6.0	8.8
		8.8	8.8	7.0	7.7	5.0	6.7	6.1	5.2	6.7
		7.0	7.0	4.0	6.1	3.1		2.2	1.1	1.0
		2.0	2.0	2.0				1.8		0.8
	Hind III	8.8	8.8	8.8	17	8.8	8.8	8.8	15	8.8
		4.1	7.3	3.8	12	4.8	3.6	7.0	8.8	4.6
		2.2	2.2	1.8	8.8	2.4	1.9	3.1	7.6	3.0
		1.4	1.4	1.0		2.0	1.2	2.0	4.0	1.7
						1.3				1.6
ECWDH6, 2.5	EcoR I	3.8	3.8	3.8	5.8	5.8	5.8	5.8	5.8	5.8
	Hind III	2.5	2.5	2.5	3.3	3.3	3.3	3.3	3.3	3.3
ECWDP7, 2.0	EcoR I	14	14	14	14	14	14	14	14	14
	Hind III	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
ECWDH8, 2.7	EcoR I	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
	Hind III	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
ECWDE9, 5.8	EcoR I	17	23	16	6.4	14	21	12	7.8	21
		13	9.0	4.4	3.5	11	18	6.4	4.3	13.5
		6.6	6.6	2.2	2.5	4.0	8.8	5.8	3.8	
		5.0	5.0			3.0	4.8	2.5	3.3	
		2.7	2.7				2.0			
	Hind III	15	12	20	11	9.7	10.3	9.2	13	9.4
		12	9.0	12	9.2	3.4	9.4	8.5	8.8	6.6
		9.0	7.8	8.1	6.7	1.5	4.2	3.4	5.5	4.2
		2.1	2.1	2.1	3.4	1.1		2.2	2.0	1.7
		1.3	1.3		2.2				1.0	

Probe Name and Insert Size	Enzyme	FSQ	FHS	FQC	BKSQ	BKFR	BKQC	WDSQ	WDCR	WDQC
ECFH10, 2.5	EcoR I	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
		2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
		2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
	Hind III	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
		3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
		2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
ECBKP11, 1.6	EcoR I	4.1	4.1	4.1	6.8	6.8	6.8	6.8	6.8	6.8
	Hind III	5.0	5.0	5.0	3.6	3.6	3.6	3.6	3.6	3.6
ECBKE12, 1.4	EcoR I	12	12	12	12	12	12	12	12	12
		2.8	2.8	4.4	1.4	5.2	6.1	1.4	4.0	6.1
	Hind III	12	12	12	12	12	12	12	12	12
		1.4	1.4	2.1	3.0	1.9	2.4	3.0	7.8	2.4
ECFE13, 2.2	EcoR I	12.5	17	12	5.2	3.4	4.0	5.4	6.6	4.0
		9.5	7.0	3.7	3.0	2.6	1.7	4.8	3.6	1.6
		5.4	5.4	1.8	2.1			2.1	3.3	1.2
		4.1	4.1						2.8	0.9
		2.2	2.2							
ECBKB14, 4.0	EcoR I	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3
	BamH I	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3
					6.3	6.3	6.3	6.3	6.3	6.3
					4.0	4.0	4.0	4.0	4.0	4.0
ECBKB15, 2.1	EcoR I	6.4	6.4	6.4	6.5	6.5	6.5	6.5	6.5	6.5
	Hind III	4.6	4.6	4.6	4.7	4.7	4.7	4.7	4.7	4.7
ECWDH16, 2.4	EcoR I	3.6	3.3	3.8	7.0	7.6	4.7	6.6	5.4	8.8
		1.9	1.9	2.1	2.7	3.0	2.5	2.7	4.4	2.5
ECWDP17, 1.5	EcoR I	3.3	3.3	3.0	5.8	5.2	3.8	5.8	3.6	3.8
		2.3	2.3	2.6			1.6		1.8	1.5
	Hind III	7.4	7.4	8.1	4.1	3.9	6.2	4.1	7.0	6.2

Probe Name and Insert Size	Enzyme	FSQ	FHS	FQC	BKSQ	BKFR	BKQC	WDSQ	WDCR	WDQC
ECWDE18, 3.1	EcoR I	16	16	16	16	16	16	16	16	16
		6.4	6.4	8.8	12.5	12	6.4	14	5.6	8.1
		4.7	4.7	3.9	3.1	3.1	3.7	3.1	3.1	2.8
	Hind III	16	16	16	16	16	16	16	20	16
		5.9	5.9	7.1	6.8	4.4	7.2	9.1	16	6.2
		1.1	1.1	2.7	1.6	1.6	5.1	5.0	6.3	1.4
					0.9			1.6	1.6	
ECBKB19, 4.0	EcoR I	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6
	Hind III	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6
		5.6	5.6	5.6						
		5.0	5.0	5.0						
ECFP20, 3.0	EcoR I	2.1	2.1	1.4	3.2	2.5	6.6	3.2	2.2	1.8
	Hind III	6.1	6.1	4.6	1.1	4.9	2.7	1.1	5.3	5.0
ECFE21, 4.6	EcoR I	4.6	1.6	1.2	1.5	6.8	6.1	1.7	5.2	4.4
	Hind III	7.4	3.8	2.6	4.1	5.3	5.5	6.2	6.0	11
						3.4			1.3	2.0
ECFE22, 2.5	EcoR I	4.8	4.8	4.8	12	12	12	12	12	12
		3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
		2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	Hind III	6.5	6.5	6.5	8.7	8.7	8.7	8.7	8.7	8.7
		4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
		3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
ECBKH23, 1.8	EcoR I	15	15	15	15	15	15	15	15	15
		8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8
	Hind III	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
		1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
ECWDP24, 1.5	EcoR I	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
	Hind III	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5

Probe Name and Insert Size	Enzyme	FSQ	FHS	FQC	BKSQ	BKFR	BKQC	WDSQ	WDCR	WDQC
ECBKH25, 3.2	EcoR I	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
		4.6	4.6	4.6	3.2	3.2	3.2	3.2	3.2	3.2
	Hind III	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
		4.6	4.6	4.6	3.2	3.2	3.2	3.2	3.2	3.2
ECBKP26, 1.2	EcoR I	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
		3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
	Hind III	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
		7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
ECWDH27, 2.2	EcoR I	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9
		3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
		2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	Hind III	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
		3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
		2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
ECFB28, 1.0	EcoR I	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7
	Hind III	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
ECWDP29, 1.4	EcoR I	0.7	0.7	0.5	7.8	10	9.0	7.8	6.3	9.0
	Hind III	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
ECBKH30, 1.8	EcoR I	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
	Pst I	3.6	3.6	3.6	6.6	6.6	6.6	6.6	6.6	6.6
		3.0	3.0	3.0						
ECWDB31, 2.0	EcoR I	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
	Hind III	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
ECFE32, 2.8	EcoR I	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
	Hind III	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
ECFH33, 2.8	EcoR I	4.2	4.2	4.2	5.3	7.4	7.7	5.3	3.9	7.7
	Hind III	2.8	2.8	2.8	7.8	10.5	9.0	7.8	6.1	9.0

Probe Name and Insert Size	Enzyme	FSQ	FHS	FQC	BKSQ	BKFR	BKQC	WDSQ	WDCR	WDQC
ECFH34, 2.4	EcoR I	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
		3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
	Hind III	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
		2.4	2.4	2.4	4.0	4.0	4.0	4.0	4.0	4.0
ECFE35, 2.9	EcoR I	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
	Hind III	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
ECFP36, 1.9	EcoR I	7.2	7.2	7.2	5.5	5.5	5.5	5.5	5.5	5.5
	Hind III	4.6	4.6	4.6	3.1	3.1	3.1	3.1	3.1	3.1
ECBKE37, 1.4	Hind III	3.5	3.5	8.4	5.0	6.8	2.6	5.0	3.0	4.4
ECWDE38, 2.0	Hind III	7.3	7.3	7.3	10	4.6	5.6	10	16	5.6
ECBKP39, 1.7	EcoR I	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
	Hind III	3.7	3.7	3.7	4.0	4.0	4.0	4.0	4.0	4.0
					3.7	3.7	3.7	3.7	3.7	3.7
ECBKE40, 2.3	EcoR I	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
		4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
		2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
ECBKH41, 1.4	EcoR I	5.9	5.9	8.7	19	13.6	12	16	21	12

Fig. 9. Autoradiograms of hybridized Southern blots used to determine genetic relatedness. Each example probe is catalogued in Table 11. A: ECBKP4, Hind III; B: ECBKB5, Hind III; C: ECWDP7, EcoRI; D: ECWDH8, Hind III; E: ECWDE9, EcoRI; F: ECFH10, EcoRI; G: ECBKP11, Hind III; H: ECBKE12, Hind III; I: ECFE13, EcoRI; J: ECFE21, EcoRI; K: ECBKH30, Pst I; L: ECWDB31, Hind III; M: ECFH33, Hind III; N: ECBKE37, Hind III; O: ECWDE38, Hind III; P: ECFP20, EcoRI; Q: ECBKB19, Hind III; R: ECBKH41, EcoRI. Sizes are in kilobase pairs.

A
20
10
6.0
4.1
2.9
1.4
1.0
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
B
17
8.8
4.1
2.2
1.4
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
C
14
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
D
3.7
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
E
16
9.0
5.0
4.0
2.5
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
F
3.6
2.8
2.2
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC

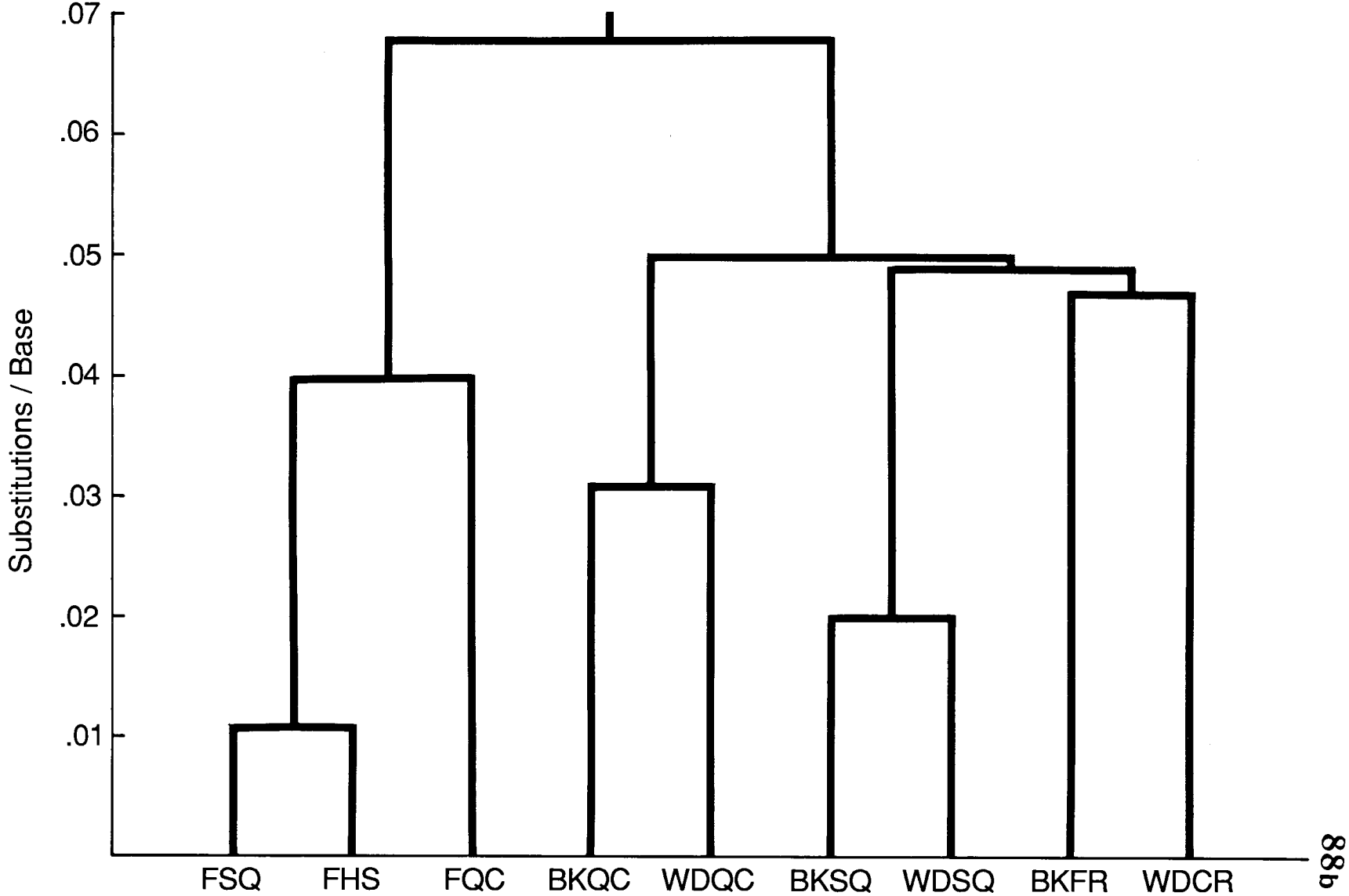
G
5.0
3.6
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
H
12
7.8
3.0
2.4
1.4
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
I
17
12
7.0
5.4
4.0
2.6
1.8
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
J
6.8
4.4
1.6
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
K
6.6
3.6
3.0
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
L
4.3
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC

— 10
— 7.3
— 5.6
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
O
— 16
— 12
— 5.9
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
R
— 8.4
— 5.0
— 3.5
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
N
— 10.6
— 5.6
— 5.0
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
Q
— 9.0
— 6.1
— 2.8
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
M
— 6.6
— 3.2
— 2.1
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
P

Table 12. Matrix of genetic distances expressed in base substitutions per nucleotide (above diagonal) and fraction of shared bands (below diagonal).

	FSQ	FHS	FQC	BKSQ	BKFR	BKQC	WDSQ	WDCR	WDQC
FSQ		.0110	.0393	.0678	.0678	.0630	.0700	.0678	.0674
FHS	.175		.0408	.0720	.0700	.0678	.0692	.0704	.0690
FQC	.484	.497		.0619	.0682	.0669	.0680	.0694	.0678
BKSQ	.673	.694	.641		.0483	.0507	.0206	.0513	.0519
BKFR	.673	.684	.675	.554		.0488	.0462	.0472	.0488
BKQC	.646	.673	.668	.571	.558		.0501	.0501	.0311
WDSQ	.684	.680	.674	.298	.539	.567		.0507	.0491
WDCR	.673	.686	.681	.575	.546	.567	.571		.0498
WDQC	.671	.679	.673	.579	.558	.410	.560	.565	

Fig. 10. UPGMA dendrogram based on the substitution data presented in Table 12. FSQ: *Fucus* population from Squamish; FHS: *Fucus*, Howe Sound; FQC: *Fucus*, Queen Charlottes; WDSQ: wood debris, Squamish; WDCR: wood debris, Campbell River; WDQC: wood debris, Queen Charlottes; BKSQ: bank, Squamish; BKFR: bank, Fraser River; BKQC: bank, Queen Charlottes.


.07
.06
.05
.04
.03
.02
.01
Substitutions / Base
FSQ
FHS
FQC
BKQC
WDQC
BKSQ
WDSQ
BKFR
WDCR
88a

animals, I found none. The example of interindividual consistency shown in Figure 11 is with the use of a probe that yields a polymorphism (between *Fucus* populations and all bank and wood debris groups) that could have been explained on the basis of half the individuals possessing one "allele" (9.5 kb fragment) and the rest of the population another "allele" (5.0 & 4.5 kb fragments). This however, appears not to be the case; all sampled animals possess both patterns. All probe/enzyme combinations were used at least twice on separate DNA samples, prepared from animals collected in different years; I saw no differences between samples.

Both sets of distance data indicated the same relative relationships. *Fucus* populations are a distinct genetic entity from bank and wood debris animals. FSQ and FHS (geographically proximal) have the most similar genotypes; BKSQ/WDSQ and BKQC/WDQC are the next most similar genotype pairs, but have very dissimilar life history phenotypes (Chapter 1). Wood debris populations are not more closely related than populations at large, indicating the wood debris life history phenotype did not arise from a colonization event by a particular ancestor or race. Instead, the life history traits typical of wood debris habitats arose independently three times through selection of independent genotypes. The fact WDSQ is more closely related to BKSQ than any other population, corroborates my suggestion from the previous chapter that WDSQ evolved from a bank population in Squamish. Other bank locations in Squamish appear to have the same genotype as BKSQ. A cursory examination of amphipod genotypes collected from several places along the east delta of the Squamish estuary (see Fig. 1, Chapter 1) indicated their genotype was the same as BKSQ (Fig. 12). This suggests that the ancestral bank population, from which WDSQ arose, also had a similar genotype to BKSQ and that the 2% divergence has occurred since the disruption of the base population's habitat

Fig. 11. A. Autoradiogram showing RFLP between BKSQ and FSQ using ECFE1, Bgl II digest; samples collected in 1984. B. Autoradiogram illustrating absence of variation between individuals collected from FSQ in 1986 using same probe /enzyme combination. Sizes are in kilobase pairs.

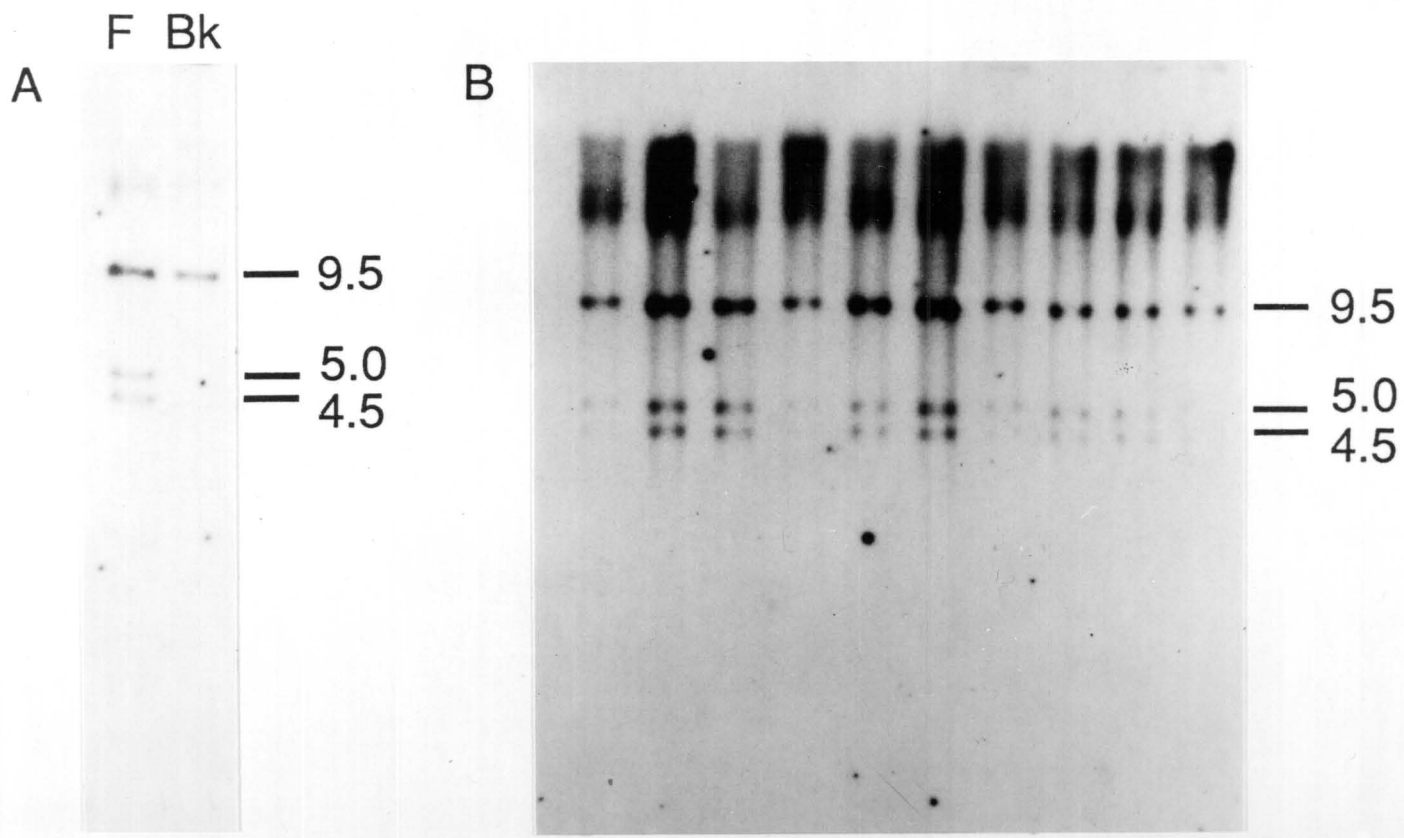
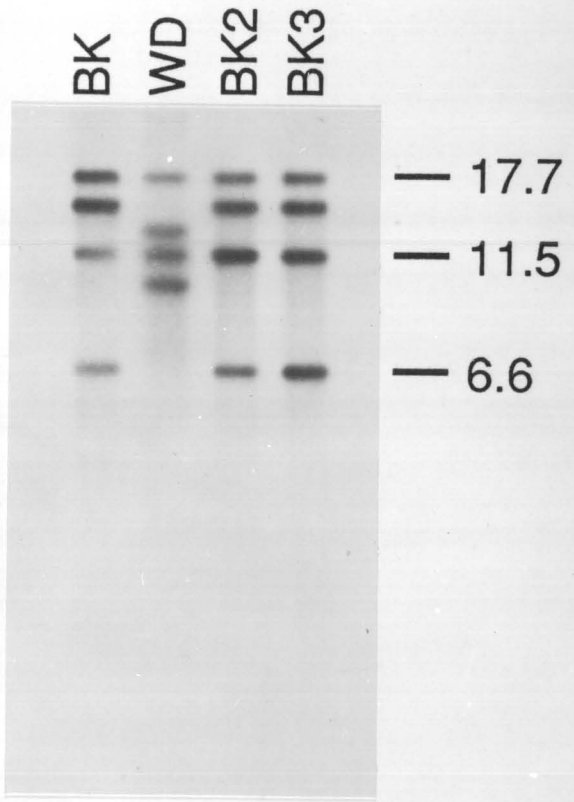

F
Bk
A
9.5
5.0
4.5
B
9.5
5.0
4.5

Fig. 12. Comparison of genotypes of BKSQ, WDSQ and two other bank populations (BK2 and BK3) along the east delta, using probe ECBKB42, Hind III digest. Purpose is to illustrate the similarity of other bank genotypes to BKSQ. Sizes are in kilobase pairs.


BK
WD
BK2
BK3
17.7
11.5
6.6

(estimated at 75 - 100 years, Chapt. 1). This also suggests that there must be gene flow between bank locations within the Squamish estuary but not across habitat types. WDQC is more closely related to BKQC than any other population, suggesting the situation in this Queen Charlotte estuary is like that in Squamish, ie. WDQC evolved from a bank population within the same estuary. I suggest that WDCR similarly arose from an unsampled bank phenotype within the Campbell River estuary. Wood debris amphipods from the three locations have very dissimilar genotypes but the same life history traits, I suggest this represents a form of intraspecific convergence or parallelism. Wood debris populations have diverged from a bank phenotype and at the same time are converging towards a wood debris life history phenotype. Parallel evolution refers to the independent evolution of a feature(s) in closely related organisms and convergence refers to homoplasy from different genetic bases. How different the genetic bases need be before it is convergence rather than parallelism is unclear and probably immaterial. Either way, the wood debris life history phenotype arose on three separate occasions and represent independent selection events; the observed variation does not reflect several colonization events by the same ancestor.

The obvious distinction in genotype between *Fucus* and the bank and wood debris populations suggests these animals may comprise a separate race, subspecies or perhaps even "hidden" species. A comparison of genotypic similarities between *Eogammarus oclari* and the nine populations, using probes such as BKH23 and WDP24 indicated all nine populations of *E. confervicolus* have conserved restriction sites which are different from those of *E. oclari* (Fig. 13). Alternatively, a probe such as BKH25 identified both conserved and different restriction sites between *Fucus* populations and bank and wood debris, which were once again different from *E.*

Fig. 13. Examples of genotypic similarities and differences between all nine populations and *Eogammarus oclari*. A: probe WDP24, EcoRI digest; B: probe ECBKH23, EcoRI digest; C: probe ECBKH25, EcoRI digest. Sizes are in kilobase pairs.

A
FSQ
FHS
FQC
BKSQ
BKFR
BKQC
WDSQ
WDCR
WDQC
E. oclari
8.0
B
15
8.8
C
7.6
4.6
3.2

oclari (Fig. 13), indicating that although *Fucus* animals are distinct from bank and wood debris amphipods, they are much more similar to other *E. confervicolus* than to *E. oclari*. I take this as evidence that whatever status one wishes to give the members of the *Fucus* ecotype it must be within the framework of the species *E. confervicolus*. Interestingly, starch gel electrophoresis of fifteen enzymes indicated that almost all loci were fixed for the same allele in BKSQ, BKFR, WDSQ, WDCR, FSQ and FHS (the Queen Charlotte populations were not sampled). Mannose-6-phosphate isomerase and glucose-6-phosphate isomerase were the only detectable polymorphisms, however, Mpi and Gpi allele frequencies were not significantly heterogenous among all six sites (J.H. McDonald, Department of Ecology and Evolution, State University of New York, Stony Brook, New York 11794 and Stanhope unpubl. data; presented in Appendix IV). In other words, starch gel electrophoresis of several enzymes did not reveal the fixed allelic differences between locations that would indicate hidden species were present. This further indicates the utility of the hybridization technique for determining relative genetic relationships in closely related groups and provides further evidence that these amphipods comprise the same species.

GENERAL DISCUSSION AND SUMMARY

This thesis documents a case of strong diversifying natural selection of life history traits in an estuarine amphipod. I demonstrate inter - population variation in life history traits, on a microgeographic scale, show that the variation has a genetic basis and that there is fitness differences associated with these variants. The presence of these three selection conditions indicates that the observed variation is the result of natural selection. Inter - population crosses indicated dominance of many of the life history traits in wood debris and *Fucus* over those typical of amphipods from bank. Of particular interest in this regard was the fact longer life span could be explained on the basis of dominant genetic effects. Analysis of genotype indicated the three Squamish populations were distinct breeding units. This was despite the fact they were only separated by as little as 300 m of intertidal mud flat and that they will form hybrids in the laboratory. The genotype of other bank locations in the Squamish estuary appeared to be identical to the principal bank location, suggesting gene flow within a habitat type but not between habitat types. The recombinant DNA techniques provided population specific markers for use in competitive ability experiments between *Fucus* amphipods and animals from bank and wood debris. The results indicated that despite the fact *Fucus* amphipods were univoltine and the bank and wood debris populations were bivoltine, the *Fucus* amphipods had a distinct overall fitness advantage in their native substrate. This suggested some form of competition may be important in the inter - habitat diversification. The fitness experiments and measurements indicated that in all cases the resident life history phenotype had on the average a fitness advantage over any immigrant life history phenotype.

The empirical testing of evolutionary theories often involves artificial selection experiments. Such efforts assess the possibility of a particular evolutionary process but often do not demonstrate a direct relevance to natural situations. I chose an approach which combines the knowledge of a natural perturbation in environment (intertidal log storage in what was once a *Carex* marsh), with a simulated version of this event (raising marsh amphipods in wood debris substrate). This allowed me to test whether a specific aspect of the perturbation was responsible for the observed differences in life history traits. Since the environmental aspect manipulated in the selection experiment differs in the food it supports (although argueably it differs in some other respects as well), it suggested a possible cause - effect relationship between low food abundance and the life history tactic of partitioning reproductive effort into fewer, larger offspring.

The selection test presented strong correlational evidence for habitat specific selection of life history traits, not only in wood debris but in *Fucus* and bank. This, combined with the fact that fitness was highest in native substrates, provides further indication of the importance of substrate as a selective agent and indicates that selection can drive life history traits to a number of local adaptive peaks. In this particular situation in fact, the selection took on an ecotypic form.

In Chapter 1 I presented evidence which indicated the *Fucus* life history phenotype was very different from the other two life history types, most significantly in age at reproductive maturity and life span; nonetheless populations from all three habitat types appeared to comprise the same species (high mating success of the F_1 and F_2). Further evidence that this pronounced diversification has taken place within an intraspecific framework, came in the genotype analysis; although *Fucus*

comprised a distinct group from wood debris and bank, they were not as distinct as the only other species in this genus.

The genotype analysis indicated that the members (populations) of the wood debris life history phenotype were not descendants of the same ancestor. In fact, WDSQ and WDQC were most closely related to BKSQ and BKQC respectively. Three different lines of evidence then (each corroborating the other), indicate the wood debris life history phenotype diverged from a bank life history phenotype: natural perturbation in the estuary (creation of a wood debris environment in what was once a bank environment) that has occurred over the last 75-100 years, selection experiment which simulated the change in estuarine environment; and the similarity of WDSQ and BKSQ genotypes. Other bank populations in the Squamish estuary appeared to have identical genotypes to BKSQ, suggesting that the bank population which formed the basis for WDSQ, was also similar to BKSQ. Independent selection events, from different ancestors, resulted in a wood debris life history phenotype in three geographically separated estuaries. This example of parallelism or convergence, has taken place over a period of about 75-100 years.

The most significant aspect of the selection experiment, lies in its simplicity: an alteration in substrate selects for an array (or suite) of life history characters. The substrate differs, most significantly I believe (and I present evidence to support this), in the amount of food it supports. I assume larger juveniles result from larger eggs and I know larger juveniles have better survivorship in wood debris substrate. I submit that the principal target of selection in wood debris habitats is juvenile size and that phenotypic correlations and possibly pleiotropic interactions between traits results in a wholesale change in life history. I offer the following hypothesis concerning the wood debris life history phenotype: selection for large juveniles

results in a drop in fecundity, because of a negative correlation (phenotypic or genetic) between the two characters, decreased reproductive output per brood, allows an increase in brood number (which should be under directional selection to increase in all habitat types) which interacts pleiotropically (positively) with life span. Interrelationships of this nature, essentially precipitating a series of changes, due to selection for one characteristic, would explain the apparent ease which one can select a wood debris life history phenotype from bank amphipods and the close correlation between traits and habitat type evident in the selection test.

APPENDIX I

BRD: brood number

DEV: development time

RESZ: size at reproductive maturity

LFSP: life span

BRDMRT: brood mortality

AGERP: age at reproductive maturity

EGSZ: egg size

EGGS: fecundity

INTCLU: interclutch interval

JUVWT: weight of newly released juveniles

JUVMRT: mortality prior to reproductive size

CLUVOL: clutch volume

ABSFIT: absolute fitness

BKSQ: bank, Squamish

BKFR: bank, Fraser River

BKQC: bank, Queen Charlottes

WDSQ: wood debris, Squamish

WDCR: wood debris, Campbell River

WDQC: wood debris, Queen Charlottes

FSQ: *Fucus*, Squamish

FHS: *Fucus*, Howe Sound

FQC: *Fucus*, Queen Charlotes

APPENDIX II

Substrate	Bacteria Per Field	Biovolume of Fungus Fragments Per Field
Wood Debris	2.7(1.8)	6.9(5.5)
Fucus	167.3(136.6)	258.8(192.1)
Bank	109.5(75.8)	86.4(68.7)

Means with bracketed standard deviations.

Biovolume is length x width.

Wood Debris: N=540 fields, approximately 100 fields for each of 5 wood chips (1cm2).

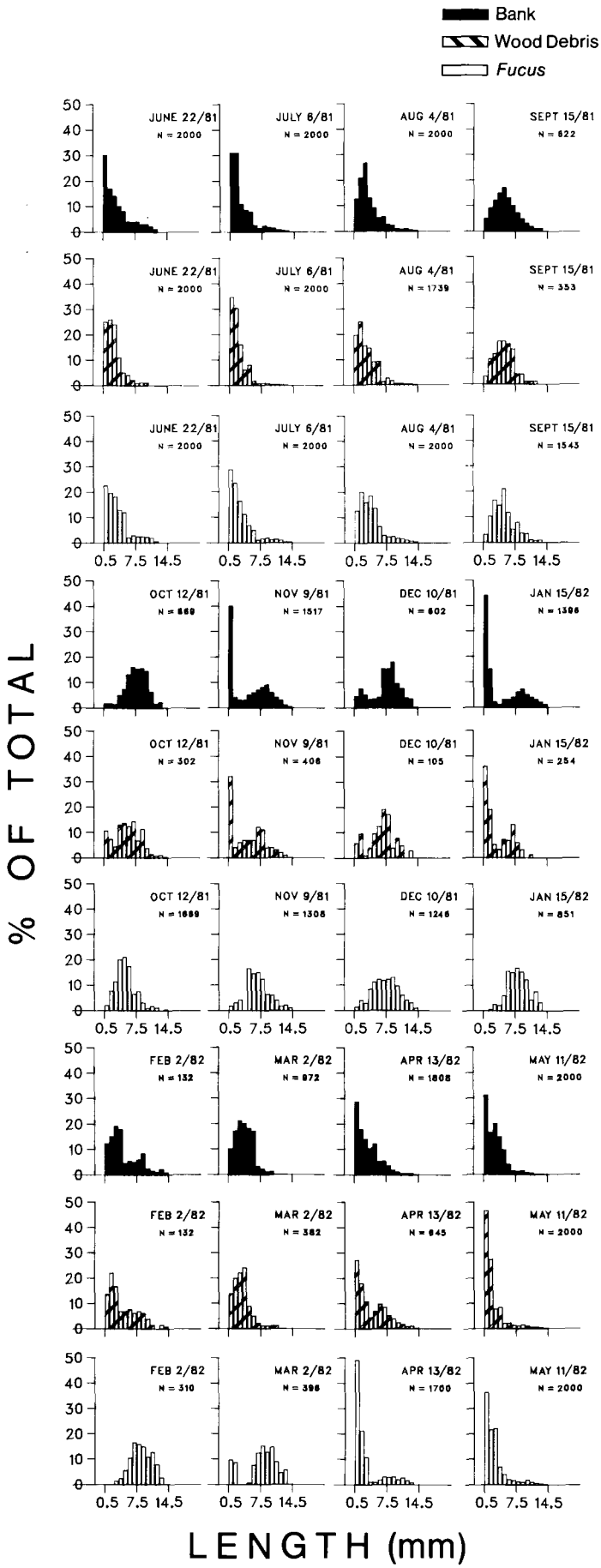
Fucus: N=80 fields from four different *Fucus* blades (1cm2 pieces).

Bank: N=50 fields from several different fragments of *Carex* rhizome (pieces 1cm long).

All samples were collected in June.

APPENDIX III

Size frequency distributions of *Eogammarus confervicolus* from the three Squamish populations over an annual period between the summer of 1981 and 82, emphasizing the univoltine cycle of amphipods in *Fucus* and the bivoltine cycle of bank and wood debris amphipods.


Bank
Wood Debris
Fucus
JUNE 22/81 N = 2000
JULY 6/81 N = 2000
AUG 4/81 N = 2000
SEPT 15/81 N = 622
JUNE 22/81 N = 2000
JULY 6/81 N = 2000
AUG 4/81 N = 1739
SEPT 15/81 N = 353
JUNE 22/81 N = 2000
JULY 6/81 N = 2000
AUG 4/81 N = 2000
SEPT 15/81 N = 1543
OCT 12/81 N = 869
NOV 9/81 N = 1517
DEC 10/81 N = 802
JAN 15/82 N = 1306
OCT 12/81 N = 302
NOV 9/81 N = 406
DEC 10/81 N = 105
JAN 15/82 N = 254
OCT 12/81 N = 1689
NOV 9/81 N = 1308
DEC 10/81 N = 1246
JAN 15/82 N = 851
FEB 2/82 N = 132
MAR 2/82 N = 872
APR 13/82 N = 1808
MAY 11/82 N = 2000
FEB 2/82 N = 132
MAR 2/82 N = 382
APR 13/82 N = 645
MAY 11/82 N = 2000
FEB 2/82 N = 310
MAR 2/82 N = 396
APR 13/82 N = 1700
MAY 11/82 N = 2000
0 10 20 30 40 50
0.5 7.5 14.5
% OF TOTAL
LENGTH (mm)

APPENDIX IV

Mpi allele frequencies:

	Location					
allele	FSQ	FHS	BKFR	BKSQ	WDSQ	WDCR
uf	.010	.000	.000	.000	.000	.009
f	.788	.779	.821	.911	.826	.759
m	.010	.010	.009	.000	.022	.009
s	.192	.202	.170	.089	.152	.213
N	52	52	56	56	46	54

Gpi allele frequencies:

	Location					
allele	FSQ	FHS	BKFR	BKSQ	WDSQ	WDCR
f	.000	.009	.000	.000	.000	.000
m	.964	.982	.982	.982	.977	.973
s	.036	.009	.018	.018	.023	.027
N	56	56	56	56	44	55

G-test for Mpi: $G=9.63$, $0.10>P>0.05$

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